

NUTRITIONAL AND PHYSIOLOGICAL STUDIES OF
REPRODUCTION IN SHEEP

By

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C O N T E N T S

	<u>Page</u>
Summary	
Introduction	1
Literature Review	4
A. Reproduction in the ewe	4
A1. The oestrous cycle	4
A1.1. Hormones of reproduction	4
A1.2. Regulation of the oestrous cycle	7
A1.3. Plasma hormone levels during the oestrous cycle	10
A2. The maintenance of gestation	17
A2.1. Gonadotrophins	17
A2.2. Steroids	19
A3. The initiation of parturition	26
A3.1. The endocrine changes at the onset of parturition	26
A3.2. Artificial induction of parturition	28
A4. The reproductive activity after lambing	30
A4.1. Lactation anoestrus	30
A4.2. The endocrine basis of anoestrus of lactation	36
A5. Hormonal control of reproduction	41
A5.1. The hormonal treatment	41
A5.2. Fertility at control breeding	44
B. The Nutritional Aspects of Reproduction	48
B1. The effects of nutrition on the reproductive cycle	48
B1.1. Oestrus	49
B1.2. Ovulation	50
B1.3. Fertility	51
B1.4. Flushing effects	52

	<u>Page</u>
B2. The effects of nutrition on the endocrine control	54
B2.1. Gonadotrophins secretion	55
B2.2. Progesterone	58
B3. The nutritional requirements of the ewe	59
B3.1. Metabolic changes in undernourished animals	61
B3.2. The assessment of the adequacy of feeding	63
B3.3. Recommended nutritional standards	66
Summary of Literature and Aims of Study	73
Materials and Methods	82
Results and Discussion	99
Chapter 1. The performance of the ewes under various planes of nutrition during late pregnancy	99
Chapter 2. The use of Dexamethasone for inducement of parturition	113
Chapter 3. The plasma concentration of progesterone, LH and prolactin during late pregnancy and induced parturition	119
Chapter 4. The effect of lactation on the resumption of reproductive activity in <u>post-partum</u> ewes	130
Chapter 5. The effect of <u>pre-</u> and <u>post-partum</u> feed intake on the reproductive activity of suckling and non-suckling ewes	141
General Discussion and Conclusion	154
References	171

I N D E X O F T A B L E S

<u>Table No.</u>		<u>Page</u>
1	Composition of diets, nutrients and digestibility	84
1.1	Details of feeding scale and the daily nutrients intakes employed during the last 6 weeks of pregnancy	100
1.2.	The daily intake, the blood metabolites concentrations and the ewes' performance during the last 6 weeks of gestation	102
1.3	Means and S.E. of blood metabolites concentrations for ewes bearing single, twin and triplets lambs within treatment	107
1.4	Effect of intake of energy and protein during gestation on ewe and lamb performance	110
1.5	Effect of energy intake on total lamb birth weight	111
2.1	Intervals between injection and parturition in ewes treated with Dexamethasone	115
2.2	Increase in weight of new born lambs after induction of parturition	116
3.1	Adjustment of feed intake according to foetal load and the ewes' performance	121
3.2	Progesterone, LH and prolactin concentration in the peripheral plasma of pregnant ewes	123
4.1	Voluntary feed intake, body weight gain and plasma FFA during the first 5 weeks <u>post-partum</u> , for lactating and non-lactating ewes	132
4.2	Body weight gain and plasma FFA during the first 3 weeks after lambing for ewes which showed oestrus and those which did not	134
4.3	The occurrence of oestrus and ovulation 35 days <u>post-partum</u> of a progestagen treatment in lactating and non-lactating ewes	136

4.4	Characteristics of the pre-ovulatory LH associated with synchronized oestrus in lactating and non-lactating ewes	137
5.1	Feed intake, body weight change and plasma metabolite concentration during the first 6 weeks after lambing for lactating and non-lactating ewes on various feed levels	144
5.2	Ovarian activity before and after synchronisation of oestrus 35 days <u>post-partum</u> in lactating and non-lactating ewes on various feed levels	146
5.3	Plasma progesterone, LH and prolactin during the first 3 weeks <u>post-partum</u> and the pre-ovulatory LH release at synchronized oestrus 35 days after lambing	149

I N D E X O F T E X T - F I G U R E S

<u>No.</u>		<u>Before Page</u>
1	Progesterone assay: standard curve	97
1.1	Mean daily energy intake and the weekly live-weight changes during the last 6 weeks of gestation	103
1.2	The weekly changes in peripheral plasma levels of glucose, FFA and ketone bodies during the last 6 weeks of gestation	105
2.1	Induction of parturition by an injection of Dexamethasone	116
3.1	Plasma progesterone, LH and prolactin concentration in late pregnancy and induced parturition	124
4.1	The relationship between body weight, FFA concentrations and oestrus during the first 3 weeks after lambing	135
4.2	The time interval between end of progestagen treatment and the onset of oestrus	137
4.3	The pre-ovulatory LH release in 3 ewes representing 1, 24 or 56 days of lactation	138
5.1	Plan of the experiment and No. of ewes	142
5.2	Body weight changes and the plasma metabolites concentration during the first 6 weeks after lambing for lactating and non-lactating ewes on different levels of nutrition	145
5.3	Plasma progesterone, LH and prolactin before and after synchronisation of oestrus in lactating and non-lactating ewes	148
5.4	Frequency distribution of plasma progesterone concentration 17 days after mating	150

LIST OF ABBREVIATIONS

ACTH	- Adrenocorticotrophic stimulating hormone
ARC	- Agricultural Research Council
CL	- corpus luteum
CP	- Crude protein
CRD	- Complete Ruminant Diet
DCP	- Digestible crude protein
DOM	- Digestible organic matter
DM	- Dry Matter
EE	- Ether Extracts
FFA	- Non-esterified Fatty Acids
FSH	- Follicle stimulating hormone
HCG	- Human Chorionic Gonadotrophin
LH	- Luteinising Hormone
LH-RH	- LH-releasing hormone
ME	- Metabolisable Energy
NFE	- Non- fat extracts
NRC	- National Research Council (U.S.A.)
OM	- Organic Matter
PGF _{2α}	- Prostaglandin F _{2α}
PMS	- Pregnant Mare Serum
RIA	- Radio-immunoassay
TDN	- Total digestible nutrients

SUMMARY

Experiments conducted in the autumn and winter months of 1971 and 1972 with a total of 98 Finn-Dorset (F_1) ewes were designed to study the effects of pre- and post-lambing feeding and the effects of lactation on the resumption of reproductive activity early post-partum. In the two years, 84 pregnant ewes were individually fed according to one of 4 different energy levels during the last 6 weeks of gestation. The number of foetuses carried by the ewe (1-4) was determined by X-ray before allocation to 5 treatments which allowed the following rations: 1) Ad-libitum feed intake (treatment 1A); 2) 33 Kcal ME/Kg/ewe live-weight + 365 Kcal ME/Kg/foetus (anticipated birth weight) (1H and 2H); 3) 80% (1M) and 4) 50% (2L) of the total energy fed to treatments 1H and 2H.

After lambing in 1971, 50 ewes, in 3 groups were maintained on a high plane of nutrition throughout, and were allowed to suckle their young for 1, 24, or 56 days. After the second lambing in 1972; 2 groups of 16 ewes had their lambs weaned on day 1 after lambing and were fed either 100% (treatment H), or reduced to 50% (R) of the energy requirements for maintenance. A third group of 13 ewes (L) suckled twin lambs and was fed ad-libitum.

During the experiments, the nutritional status of the ewes was monitored once a week by weighing, and by determination of plasma metabolites (glucose, non-esterified fatty acids (FFA) and ketone bodies) in blood samples collected weekly.

In late pregnancy adjustment of feed intake to foetal number standardised the nutritional state within treatments and a uniform body condition was achieved irrespective of litter size. The FFA as a measurement of energy metabolism, differed significantly even between high energy treatments but not between single, twin or triplet bearing ewes within treatments. It was therefore concluded that their use may not necessarily be limited to the indication of undernutrition only.

The live-weight changes of the ewe failed to reflect the short term nutritional differences in the last 6 weeks of gestation and all the treatment groups gained 6-8 Kg during this period (except 2L which remained constant) and lost 8-10 Kg at parturition. The results indicated that the adverse effects of nutritional deficiencies during late pregnancy could be overcome by good nutrition during the first 100 days of gestation.

The lamb birth weight was not affected by the wide range of energy (2.3-4.4 Mcal ME/ewe/day) fed on a constant level during the last term of gestation, indicating the limited use of lamb birth weight as an index of adequacy of nutrition in pregnancy.

Plasma progesterone, Luteinizing hormone (LH) and prolactin were measured on day 100, 120 and 134 of gestation and on the day of parturition in two treatments 2H and 2L. On the 100th day of gestation before allocation to treatment groups, litter size affected the concentration of plasma progesterone ($P < 0.001$), but had no effect on days 120 or

134 of gestation when the ewes were fed according to litter size. The low feed intake, however, caused a significant increase in plasma progesterone concentration. Thus, it is possible that the effect of litter size on plasma progesterone concentration recorded on day 100 of gestation was partially mediated via nutrition. These results indicate the possible use of plasma progesterone for pregnancy diagnosis and for the assessment of the nutritional state of pregnant ewes.

Lambing was induced in the ewes of treatments 2H and 2L by a maternal injection of dexamethasone (16 mg i.m.) on day 141st of gestation and parturition occurred 49.0 ± 2.18 hours after the injection, with 90% of the injected ewes lambing by the 143rd day of gestation compared with the 147th day in the control group. There was no ill effect on the ewe or the lamb born and the plasma progesterone values measured within hours after parturition were below 1 ng/ml as in normal delivery.

LH and prolactin concentrations showed no major change during late pregnancy, although there was a very high increase in the prolactin concentrations at the time of parturition. No effects of nutrition or litter size was observed.

During the post-partum period, between 2 and 20 days after lambing, spontaneous oestrus was observed in 8/17 dry ewes in 1971 and in 11/16 (H) and 8/16 (R) dry ewes in 1972 compared with 1/33 and 1/13 (L) lactating ewes for the 2 periods, respectively. In the peripheral blood samples, taken every 3-4 days, the mean plasma hormone concentrations for the

3 weeks period were: progesterone: H, 0.50 ± 0.09 ;
R, 0.44 ± 0.09 and L, 0.22 ± 0.10 ng/ml. LH: H, 1.78 ± 0.44 ;
R, 1.45 ± 0.44 and L, 1.04 ± 0.48 ng/ml. Prolactin: H, 102 ± 43 ;
R, 29 ± 43 and L, 80 ± 44 ng/ml.

Progestagen treatment (vaginal sponges) inserted from days 21-33 post-partum increased the plasma circulating level of progesterone and suppressed LH concentration to 0.2 ng/ml in all the animals. The plasma prolactin levels were not affected.

Following the progestagen treatment all the ewes in the first year and most of them in the second year showed oestrus. The interval between sponge withdrawal and the onset of oestrus was, however, longer in the lactating ewes than in the dry ones (41.0 ± 1.5 vs. 35.3 ± 1.5 hrs., $P < 0.01$ and 39.3 ± 2.0 vs. 35.5 ± 1.9 NS, respectively). Delay was also observed in the start of the pre-ovulatory LH release after onset of oestrus (12.6 ± 1.3 vs. 9.1 ± 1.2 , $P < 0.01$ and 8.0 ± 1.3 vs. 5.0 ± 1.2 NS, respectively). Ovulation, was not adversely affected by lactation. The nutritional state of the ewe had no effect on oestrus, ovulation or the hormone levels during the oestrous cycle following synchronisation. The progesterone levels measured on day 17 after mating also indicated high conception rates (12/13, 11/15 and 9/11 ewes mated from H, R and L treatments, respectively). However, the actual number of ewes lambing was much lower, the reduction being greatest in the underfed ewes. Only 1/15 R ewes lambed compared with 6/13 H and 5/11 L ewes diagnosed to have conceived.

INTRODUCTION

The number of viable lambs produced by the ewe in a given period of time is one of the main factors affecting the biological (Newton, Betts and Large, 1970) and economic efficiency (Donald, Read and Russell, 1970) of meat production from sheep. Theoretically, the ewe, having a gestation period of 147 days, could lamb twice yearly. However, this is not the normal breeding pattern of sheep, which lamb only once a year. Generally sheep have only one restricted breeding season during the year, the duration of the season varying to some extent according to the breed and latitude (Hafez, 1952). There is a growing interest and a need for improving the efficiency of rate of reproduction in sheep which was recognised by Hammond (1957) who discussed the need for fat lamb production systems that are less seasonal in character, as well as the possibility of increasing the number of lambs born per ewe.

Besides the application of hormone treatment, light control and the manipulation of the genotype that have attracted much research, a production system that has generated a lot of interest is the programme termed "accelerated lambing". The programme is designed to shorten the interval between lambing to less than one year; this means that the post-partum interval to the recommencement of sexual activity must be reasonably short so that the ewes may become pregnant again with a minimum of delay.

The importation of the highly prolific Finnish Landrace sheep to Britain has provided one potential means of increasing

reproduction. The breed is reported to have a mean litter size of 2.5-3.0 lambs under good management conditions (Donald and Read, 1967). Crossing this breed with the Dorset Horn, which has a long breeding season of about 7 months compared with most British breeds (Newton and Betts, 1967), has resulted in a substantial increase in the number of lambs born and raised per ewe (Donald, Read and Russell, 1968) and a longer breeding season, which will enable the introduction of a twice-yearly lambing system.

In developing such a system of frequent lambings, additional and more exact information is needed on several aspects of sheep reproduction:

1. The percentage of ewes which are able to conceive in different 'out-of-season' months, the heritability of this ability and its reflection in the physiological state and hormonal control of reproduction.
2. The type of management and husbandry that are needed to induce a reliable high proportion of ewes to take the ram and to conceive within 5 weeks after lambing.
3. The effect of lactation and the importance of weaning lambs on the new conception after different post-partum periods.
4. The extent to which synchronisation of heats and lambing can be obtained and the optimum time for mating and insemination.
5. Out of the management factors which could be of great importance in ensuring the success of more frequent lambings, the role of nutritional factors in post-partum

returns to oestrus and fertility deserve special mention. If more frequent lambing is to become more widely adopted, greater attention will have to be paid to nutrition between parturition and re-mating, whether the ewe is lactating or not.

In the present study the physiological and nutritional aspects of reproduction will be examined more closely, with a special emphasis on the effect of suckling on the resumption of reproductive activity after lambing, and how this is influenced by the nutritional level during the pre- and post-partum periods.

LITERATURE REVIEW

A. REPRODUCTION IN THE EWE

A1. The Oestrous Cycle.

A1.1. Hormones of reproduction

a. Gonadotrophins:- Fevold, Hisaw and Leonard (1931) were the first to recognise the dual nature of the pituitary gonadotrophic secretion on reproduction, and separated the pituitary extracts into two active fractions: follicle-stimulating hormone (FSH) and luteinising hormone (LH). Later pure FSH and LH preparations were made from sheep and pig hypophyses (Li, 1949), these were identified as glyco-proteins.

FSH stimulates the growth of the ovarian follicle by promoting mitotic proliferation of the granulosa cells. The response to FSH is demonstrable by the presence of follicles in all stages of development, initiation of antrum formation and increase of ovarian weight. LH is now well accepted to be responsible for stimulation of steroidogenesis in the ovarian follicle and for ovulation in the ovary previously stimulated by FSH, as well as the transformation of the Grafian Follicles into Corpora Lutea (CL) (Keyes, 1969). During ovulation the effects produced by LH include expansion and maturation of the Grafian Follicle until the ovarian wall ruptures; thereby releasing the ovum together with some viscous follicular fluid and cells. After ovulation, under the influence of LH, some granulosa cells increase in size and undergo the process of luteinisation.

Dresel (1935) was the first to observe that prolactin

might have an action on the ovary of the mouse, and Lahr and Riddle (1936) later extended these observations to the rat. Astwood (1941) was able to show that the life span of ~~corpus~~ **L**uteum of the rats could be prolonged by prolactin injections. Thus prolactin came to be known as the luteotrophic hormone. There is now growing evidence to suggest that prolactin may have a luteotrophic action in sheep (Denamur, Martinet and Short, 1972), but the role of prolactin and its changes during the oestrous cycle in the ewe remains to be clarified.

b. The Regulation of the Gonadotrophins Secretion:-

The development of modern ideas on neurohormonal control of the anterior pituitary gland may be traced back to the theory of Hinsey and Markee (1933), that hypothalamic stimuli activated the posterior lobe of the pituitary, which in turn influenced the anterior lobe by hormonal pathways. In the same period, the work of Marshall (1936), indicated that the central nervous system was involved in the control of secretion of the gonadotrophic hormones of the pituitary gland. The first direct evidence for the existence of a hypothalamic neurohormonal regulating LH release was provided independently by McCann, Taleisnik and Friedman (1960), by Harris (1961) and by Campbell, Fewer, Garcia and Harris (1961), who showed that hypothalamic extracts of at least seven animal species are capable of stimulating LH release.

Reeves, Arimura and Schally (1971) showed that after administration of purified porcine LH-RH into ewes, an elevation of serum LH occurred at all stages of the oestrous

cycle. However, the same dose of LH-RH induced a greater change in serum LH during a 4 to 12 hour period on day 1 of the oestrous cycle and may be an important factor in the preovulatory surge of LH. Recent studies indicate that administered synthetic LH-RH caused elevation of plasma LH in ewes and that it could also induce ovulation (Arimura, Debeljuk, Matsuo and Schally, 1972).

Gonadotrophin secretion is also influenced by the gonadal steroids, both stimulatory and inhibitory. Arimura and Schally (1971) suggested that oestrogen may potentiate the responses to LH-RH in rats and sheep at the pituitary level. These results are in agreement with those of Weick, Smith, Dominguez and Dhariwal (1971), who argued that oestrogen decreases the pituitary threshold to LH-RH.

Progesterone can either stimulate or inhibit the release of LH depending upon the time of administration during the oestrous cycle (Everett, 1961). Caligaris, Astrada and Taleisnik (1968) also showed that progesterone has a biphasic effect, first stimulating and then inhibiting the release of LH. It has been suggested that progesterone may facilitate LH release in rats by lowering the hypothalamic activation threshold to neural stimuli (Döcke and Dörner, 1969).

The LH and FSH secretion is thus held in a reciprocal relationship with sex steroid secretion. When sex steroid secretion is reduced (e.g. by castration), the secretion of LH and FSH is increased. Oestrogen by itself can inhibit LH release in castrated rats (Schally, Bowers, Carter, Arimura, Redding and Saito, 1969). However the combination

of oestrogen with progesterone appears to be more effective. The work with synthetic progestagens suggested that the site of negative "feedback" of progestational steroids may be principally in the hypothalamus (Schally, Parlow, Carter, Saito, Bowers and Arimura, 1970). However, the recent evidence of Arimura and Schally (1970) indicates that in the rat and rabbit progesterone exerts some of its inhibitory effects directly on the pituitary.

Al.2. Regulation of the Oestrous Cycle

a. The Luteal Phase:- Edgar and Ronaldson (1958) were among the first to show that the sheep's CL attains full secretory activity by about the 6th and 8th day of the oestrous cycle and continues secreting progesterone at a fairly constant rate until about the 15th day. A report by Short, MacDonald and Rowson (1963) indicated that in addition to progesterone a low level of oestradiol 17 β was produced by the ovary during the luteal phase of the sheep's oestrous cycle. Studies by Baird, Goding, Ichikawa and McCracken (1968), in sheep with ovaries transplanted to the neck, also revealed the presence of oestradiol 17 β in minute amounts in blood draining ovaries containing an active CL.

The data of Short (1964) and Thorburn, Bassett and Smith (1969) indicated some decline in progesterone output between the 10th and 15th day. Electron microscopy studies also yielded some evidence of incipient regression commencing as early as day 12 to 13 of the cycle (Dean, Hay, Moor, Rowson and Short, 1966). In their view, the sudden decline

suggested an active inhibition of progesterone secretion, and much work has been concentrated on the factors involved in corpus regression. In a review Rowson (1970) concluded that evidence exists for an endometrial luteolytic effect. Thus uterine luteolysin could be a prostaglandin ($\text{PGF}_{2\alpha}$) which was found to cause a sudden substantial fall in progesterone secretion within a relatively short time of administration (Barrett, Blockey, Brown, Cumming, Goding, Mole & Obst, 1971).

b. Preoestrus:- At the end of the oestrous cycle, the progesterone declines rapidly to a negligible level and this situation remains during oestrus and until fresh CL is produced at ovulation. Robertson (1967), in discussing data for gonadotrophin release in the cyclic ewe, estimated a possible interval of about 36 hours between corpus regression and release of the pituitary FSH. ~~Oestrus~~ began about 12 hours after this release. This timing would agree with data for sheep treated with prostaglandin ($\text{PGF}_{2\alpha}$) which showed ~~oestrus~~ about 48 hours after induced regression of the CL (Barrett et al., 1971).

The decrease in progesterone is presumably important in initiating the release of a preovulatory surge of FSH, which is believed to occur several hours before the onset of oestrus (Robertson and Rakha, 1966) and is followed by a rapid build-up of an ovarian oestradiol secretion.

c. Oestrus:- Oestrus is the behavioural response of the ewe to this oestrogen; the steroid reaching its peak at about the start of the heat (Cox, Mattner, Shutt and Thorburn, 1971), after which it disappears rapidly from the circulation.

As well as bringing about oestrus in the ewe, oestrogen is known to be primarily responsible for inducing the release of the preovulatory surge of LH which occurs in the first 12 hours of heat (e.g., Geshwind and Dewey, 1968; Niswender, Roche, Foster and Midgley, 1968; Wheatley and Radford, 1969; Goding, Catt, Brown, Kaltenbach, Cumming and Mole, 1969). In an earlier study, Robertson and Rakha (1966) reported that the FSH content of ovine pituitary began to decrease 8 hours before the onset of oestrus by which time 28% of the FSH content of the pituitary had been discharged. The discharge of FSH continued at a steady rate until 6 hours after the onset of oestrus to result in a further decrease of 24%.

No change in the LH content of the pituitary occurred during the 12 hours before the onset of oestrus. The discharge of LH started at the onset of oestrus and was completed within 6 hours by which time 52% of the LH in the pituitary had been released. Radford, Wallace and Wheatley (1971) reported that the release of LH in turn stimulates the nuclear maturation of the oocyte and rupture of the follicle in those follicles previously sensitised by FSH. Ovulation occurs about 1 day after LH release (Cumming, Brown, Blockey, Winfield, Baxter and Goding, 1971), and its timing should usually coincide with the end of oestrus. This agrees with the data on the occurrence of ovulation after HCG injection in the ewe in early oestrus (Dziuk, 1965) and with the completion of nuclear maturation in the sheep oocyte cultured in vitro (Crosby and Gordon, 1971).

The observed reduction in the pituitary content of FSH and LH preceding ovulation in sheep (Dierschke and Clegg, 1968; Robertson and Rakha, 1966) clearly imply that both hormones are involved in bringing about this event, but it is necessary to know much more about the precise influence of the sheep's CL on the release of the pituitary gonadotrophins, both in terms of the amount of FSH/LH released and the timing of this release (relative to the regression of CL). In the case of LH there is evidence that the two levels of secretion (basal and preovulatory) are involved, each under the control of a separate centre in the hypothalamus. The basal level is apparently maintained in the face of luteal activity whereas the preovulatory surge is blocked by progesterone but released in response to oestrogen at the time of heat (Goding et al., 1969). In the case of FSH, it is still uncertain whether there is a basal level of secretion in addition to a main preovulatory surge.

A1.3. Plasma Hormone Levels during the Oestrous Cycle

The observations reviewed in section A1.2 regarding pituitary secretion seem to provide acceptable criteria of hormone release, but determination of ^{the} precise time and interval of gonadotrophin release must be confirmed by quantitative analysis of blood hormones. Information is now accumulating rapidly with the advent of appropriately sensitive assay procedures for dealing with gonadotrophins and sex steroids. Attempts have been made to study how these changes in the pituitary gonadotrophin levels, as

assessed by sacrificing groups of animals at different stages of the reproductive cycle, reflect concomitant changes in the blood levels. These were assayed by extracting gonadotrophin from the urine (see review by Robertson, 1969), and in the peripheral blood by the radio-immunological assays developed recently.

a. Luteinising Hormone (LH):- Using radioimmunoassay (RIA) techniques several authors were able to measure the levels of LH in the ovine plasma at different stages of the oestrous cycle. Results of the same order were reported by e.g. Niswender et al. (1968),^{and} Pelletier, Kann, Dolais and Rosslin (1968). Using the solid-phase RIA method (Catt and Tregear, 1967), Goding, et al. (1969), reported a mean resting concentration of 3 ng/ml of plasma, with an abrupt surge of LH commencing 4 to 16 hours after oestrus was first detected. In each case the concentration had returned to the basal level by 10 hours after the start of the surge. The highest value found was approximately 200 ng/ml and the plateau lasted for less than 2 hours. To observe the peaks, the necessity for frequent sampling was stressed.

Peaks of the same magnitude and duration of those observed during the normal cycle were obtained after administration of oestradiol 17 β to anoestrous sheep (Goding et al., 1969). On the other hand, progestagen treatment without, or with, PMS was found to affect significantly the secretion of the peak LH level by Pelletier and Timonier, (1969). They reported that the mean levels of LH released were 137 ± 14 ng/ml for non-treated ewes compared with 78 ± 8

and 99 ± 14 ng/ml for the two treatments respectively. The duration of the discharge (12 hours) was not affected. LH peaks similar to those obtained in the treatment groups were, however, reported by Pant, Hopkinson and Fitzpatrick (1972) for 6 untreated Clun Forest ewes.

b. Prolactin:- Basal circulating levels of prolactin in ruminants were first measured by Johke (1969) who took daily blood samples from heifers and goats, and found the average basal circulating levels to be 2.0 ± 1.6 ng/ml and 6.8 ± 5.2 ng/ml respectively, with a considerable day to day variation in the levels of prolactin. A wide variation in plasma concentration levels during the day in lactating cows, heifers and bulls was also reported by Schams and Karg (1970) and Swanson and Hafs (1971).

Reeves, Arimura and Schally (1970) studied the variation in blood prolactin and LH measured simultaneously during various stages of the oestrous cycle in the ewe. They reported that prolactin levels were significantly higher during preoestrus - day 14 until oestrus (49 ng/ml) - and day 1 of oestrus (40 ng/ml) than during day 2 of oestrus (11 ng/ml), meteoestrus - day 3 and 4 (15 ng/ml) - and dioestrus - days 5 ^{to} through 13 (19 ng/ml). LH levels were elevated on day 1 of oestrus (56 ng/ml) and low in the other stages, levels ranged between 1.4 to 1.9 ng/ml. These data suggest that the duration of the rise in prolactin levels is longer and precedes the elevated LH level at oestrus. In this experiment serum prolactin levels were not correlated with the rise in LH, even though the increase in LH occurred

at a time when serum prolactin levels were still high.

The results of plasma prolactin measurements reported by Kann (1971) and Bryant, Greenwood, Kann, Martinet and Denamur (1971) showed that massive prolactin secretion occurs at oestrus in the ewe. Serum levels of prolactin measured at 3 or 4 days were also reported to increase during the cycle, and a peak prolactin level was noted on the day of oestrus very near the time of the ovulatory release of LH (Davis, Reichert and Niswender, 1971). Similar results were reported by Cumming, Brown, Goding, Bryant and Greenwood (1972) who studied the release of prolactin in more detail over a 47 hour period from the onset of oestrus in the ewe. Surges of prolactin occurred around the time of preovulatory LH release which began to rise 4 to 16 hours after oestrus was first detected. The prolactin concentration reached its peak (in one case 140 ng/ml) during the first 10 hours after the onset of LH peak, but no large peaks were observed after the prolactin concentration returned to base line. This pattern of secretion raises the possibility that there could be a common mechanism causing the release of these two hormones. This was confirmed by the finding of Fell, Beck, Brown, Cumming and Goding (1972) who reported that oestradiol 17 β administered to anoestrous ewes caused, after a 5 to 10 hour interval, the release of prolactin as well as LH. However, this is probably not the sole mechanism responsible for prolactin release, as prolactin appears to be much more readily secreted than LH under conditions of psychological disturbances.

There is considerable evidence to suggest that when

considering basal levels of prolactin in mammals, particular attention should be paid to minimising the effects of undue stress on the animal. Evidence that stress might cause prolactin release in the ~~goat~~ was provided by Bryant, Linzell and Greenwood (1970), who noted that the stress associated with holding the goat on its side caused a large increase in the blood prolactin after 2 minutes. Holding of ewes, and in some instances copulation, was shown to be followed by a sudden increase of prolactin concentration (Cumming et al., 1972).

c. Oestrogens:- Moore and Brown (1967) carried out a very detailed time study of oestrogens (oestradiol 17 β and oestrone) and progesterone levels in ovarian venous blood of cycling ewes by a chemical fluorometric method. The level of oestradiol 17 β began to rise from the level of 10 to 30 pg/ml at -24 hours to reach a peak of 1000 pg/ml between -7 and 0 hours from oestrus. This value then dropped rapidly to around 100 pg/ml by +8 hours and was back to 10 to 30 pg/ml by the time of ovulation. Oestrone followed the same pattern but at a much lower level; the peak value was found around the time of oestrus to be 50 pg/ml. In sheep, oestrone makes up less than 10% of the oestrogens identified.

Norman, Eleftheriou, Spies and Hoppe (1968) measured free plasma oestrogens during the cycle. The total oestrogens level was found to increase from 1.4 ng/ml on the 3rd day to the value of 25.3 ng/ml on the 15th day. A sample taken on the 1st day gave a value of 8.4 ng/ml. A similar pattern of secretion was reported by Moore, Barrett, Brown, Schindler, Smith and Smith (1969).

d. Progesterone:- The functional activity of the CL in terms of its ability to secrete progesterone was first reported by Edgar and Ronaldson (1958) who were able to measure the concentration of progesterone in the venous blood of the ovary containing the CL. Their findings were confirmed and extended by Short (1964) and Dean et al. (1966). The main finding is the concentration of progesterone in ovarian venous and in peripheral blood rises gradually from the time of ovulation to a peak, on or about the 8th day of the cycle. The progesterone level plateaus out and remains constant until some time between 60 and 30 hours before the onset of the next oestrus. At this time there is a marked fall in the progesterone secreted from the CL and this is paralleled by a sharp decrease in the concentration of progesterone in peripheral blood.

The daily rate of secretion of progesterone by the ovary has been estimated during the oestrous cycle as 3.3 to 7.4 mg (Short et al., 1963). Similarly, Karsch (1970) reported that the concentration of progesterone in luteal tissue increased from a non-detectable level at oestrus to 31 $\mu\text{g/g}$ at Day 9 of the oestrous cycle, and remained at this level until Day 15 when it fell abruptly. Moore and Brown (1967) reported that in ovarian venous blood of cycling Merino ewes progesterone levels rose from a level of 10 to 20 ng/ml of plasma during the first 24 hours to a peak of 1 to 1.2 $\mu\text{g/ml}$ by the 8th day. This level was maintained until about 48 hours before the next oestrus when the level was 200 to 400 ng/ml. Twenty hours later, the level was generally in

the 0 to 1 ng/ml range.

In the peripheral plasma, Robertson and Sarda (1971) reported that progesterone concentration was very low, 0.1 to 0.2 ng/ml on the day of oestrus, and did not increase until the 4th day of the cycle. A peak concentration of 1.50 to 2.50 ng/ml was reached about the 11th day, and started to drop sharply on the 14th day to the value of 0.1 ng/ml at the onset of the next oestrus. Similar progesterone concentration in the peripheral plasma was also reported by Stabelfeldt, Holt and Ewing (1969), Thorburn et al. (1969) and Pant et al. (1972).

A2. The Maintenance of Gestation

A2.1. Gonadotrophins

The gonadotrophic hormones, both from pituitary and the placenta have an important function in promoting the synthesis of the essential steroid hormones, oestrogen and progesterone, in the ovary of the pregnant ewe. The pituitary hormones are essential for converting the CL of the cycle into one of pregnancy (Nalbandov and Karsch, 1968). The major components of the pituitary luteotrophic stimulus consists of prolactin and LH (Denamur et al., 1972). No detailed measurements of gonadotrophins during pregnancy in the sheep have been reported. Some data on pituitary gonadotrophin levels in ewes about 100 days of pregnancy have been published by Robertson and Hutchinson (1962), in which the pituitary FSH levels were similar to those found during the luteal phase of the cycle and a relatively low level of LH was noted. The steady decrease in the number and size of ovarian follicles after about the first month of pregnancy in the ewe (Hunter, 1959) suggests that the rate of synthesis and/or release of the gonadotrophins may gradually be reduced during pregnancy. This was confirmed in recent studies using radioimmunoassay techniques.

a. Luteinising Hormone (LH):- Niswender et al. (1968) reported that LH levels in ewes during the first 20 days, and at Day 40 of gestation were very low <0.6 ng/ml or non-detectable. Goding et al. (1969) found no rise in plasma LH during pregnancy, if anything a slight fall occurred, the mean value in 25 pregnant ewes being 1.9 ± 1.0 (SD) ng/ml.

They concluded that the placenta of sheep does not produce a hormone analogous to ~~Human~~ **Chorionic** gonadotrophin (HCG). Low levels of gonadotrophins in the blood of pregnant ewes were also reported by Geschwind and Dewey (1968) and Davis et al. (1971).

b. Prolactin:- Although prolactin has been suggested to have luteotrophic properties in the hypophysectomised, hysterectomised ewe (Denamur and Mauléon, 1963), further study showed that the CL of gestation is maintained without observable increase in the level of prolactin in the blood of pregnant ewes. In a preliminary study, Arai and Lee (1967), using radioimmunoassay procedure to measure prolactin during pregnancy, suggested that the level of prolactin fell as pregnancy progressed. Similarly, Davis et al. (1971) reported that serum prolactin stabilised at a low level (2.4 to 6.0 ng/ml) during months 3 and 4 of pregnancy, appeared gradually to increase 3 to 5 weeks prior to parturition, then increased rapidly three days before parturition. The highest levels (402 ng/ml) were noted on the day of parturition. McNeilly (1971) reported two peaks in the concentration of prolactin during pregnancy in sheep; one occurred 5 weeks before, and the other 1 to 7 days before parturition. Further evidence has been provided by Fell et al. (1972) who found high levels of prolactin before and after parturition in sheep. The jugular prolactin levels increased from 50 to 400 ng/ml over the final 3 days of pregnancy.

A detailed study of the changes in progesterone, corticosteroids, oestrone, oestradiol 17 β , LH and prolactin

in maternal plasma around parturition has been reported by Chamley, Buckmaster, Cerine, Cumming, Goding, Obst, Williams and Winfield (1973). In this study, LH levels remained relatively constant (2.0 to 6.0 ng/ml) from 36 hours before to 36 hours after parturition, but the prolactin levels rose sharply over this period. The prolactin levels measured in samples taken on Day -3 were in the range of 2.0 to 29.0 ng/ml, while much higher concentrations (100 to 640 ng/ml) were measured immediately before parturition. Fell et al. (1972) showed that a release of prolactin occurs in association with a surge of LH resulting from infusion or intramuscular injections of oestradiol 17 β to anoestrous ewe. On the basis of this evidence it is tempting to suggest that elevation in circulating oestrogen levels may, in part, be responsible for the observed release of prolactin at parturition, while the lack of LH release at the same time could be because of low pituitary content of LH at that time (Chamley et al., 1973). However, prolactin was found to be more readily secreted than LH under conditions of stress. Rapid rises in plasma prolactin were reported in goats after stress, sexual excitement or mating (Bryant et al., 1970). Other workers have also suggested that the venipuncture and other stressful conditions may influence the levels of prolactin in serum (Bryant and Greenwood, 1968).

A2.2. Steroids

a. Oestrogens:- Extracts of CL were superior to crystalline progesterone in maintenance of pregnancy in ovariectomised rabbits, implying that some other compound in

the CL, possibly oestrogen, was synergistic with progesterone in maintenance of pregnancy (Allen, 1937). Oestrogens seem to act not only on their target organs but also, indirectly, through their effect on other hormones. Oestrogens are generally believed to have important functions in mammalian gestation by virtue of a variety of anabolic and metabolic effects with the growth of the uterus, and more specifically with the synthesis of protein of the contractile mechanism, and of enzymes concerned in an energy provision to the foetus (Segal and Scher, 1967).

Until recently, the quantification of oestrogen has been difficult to assess because of a lack of a specific and sensitive assay capable of measuring the extremely low levels present in the blood. Challis, Harrison and Heap (1971) measured the total unconjugated oestrogens in jugular venous blood in 5 pregnant sheep by radioimmunoassay. The oestrogen levels were less than 20 pg/ml for most of pregnancy, rose to 40 to 50 pg/ml within 5 days of parturition and increased very sharply to much higher levels during the last 48 hours of gestation. In the uterine vein blood on Day 102 the oestrogen concentration was very low, less than 5 pg/ml and increased to about 100 pg/ml by Day 139. During the last 48 hours there was an increase from 400 pg to a maximum of 2600 pg/ml 5 hours before parturition, declining again to less than 500 pg/ml 4 hours after delivery. Thin layer chromatography (TLC) showed that oestrone accounted for the largest proportion of the total oestrogens. An acute increase in the rate of rise of the plasma oestrogens occurs over the last few days of gestation (Challis, 1971; Thorburn,

Nicol, Bassett, Schutt and Cox, 1972; Robertson and Smeaton, 1973). It has been suggested that these changes may comprise an important factor in the onset of the parturition process (Bedford, Challis, Harrison and Heap, 1972). The relationship between the maternal plasma levels of cortisole, progesterone, oestrogens and the initiation of parturition in the sheep and in the goat has been reviewed by Liggins, Grieves, Kendall and Knox (1972), and by Bedford et al. (1972).

b. Progesterone:- The work of Fraenkel(1903) showed that CL were necessary for maintenance of pregnancy in the rabbit. Allen and Corner (1929) demonstrated that extracts of CL could maintain pregnancy in the ovariectomised rabbit. The active substance from the CL i.e. progesterone, was isolated and chemically identified by Butendandt, Westphal and Cobler (1934). Moore and Rowson (1966 a,b,c,d) showed that an embryo must be present in the uterus on Day 12 of the oestrous cycle if the CL is to be maintained. It was also known that the CL is necessary for the maintenance of pregnancy during the first 50 days of gestation (Denamur and Martinet, 1955) in the sheep.

Ovarian and Placental Progesterone:- The concentration of progesterone in the ovarian vein in pregnant ewes has been found to be similar to those in the luteal phase ranging between 1 and 2 $\mu\text{g/ml}$ throughout gestation with considerable variation between ewes (Edgar and Ronaldson, 1958; Short and Moore, 1959; Lindner, Sass and Morris, 1964), so whatever the nature of the luteotrophic stimulus, it seems that it is the life span rather than the secretory activity of the gland

that is increased. The difference between the relatively stable level of progesterone in plasma of the ovarian vein throughout gestation, and the shift in the concentration of progesterone in the peripheral plasma, can be attributed to secretion of progesterone by the placenta. Linzell and Heap (1968) found that at Day 125 the ovary secreted 2 mg/day of progesterone while the placenta secreted 14 mg/day.

More recent chronic observations on ewes in which uterine and ovarian venous blood has been diverted into the mammary vein (Thorburn and Mattner, 1971) have shown that the placental progesterone output of single and twin bearing ewes rose from about 4 mg and 8 mg respectively at Day 100 to about 30 mg and 56 mg respectively 5 days before parturition, compared with the maximum production rate of 100 mg/day determined from the metabolic clearance rate of continuously infused isotopically labelled progesterone in pregnant sheep by Slotin, Harrison and Heap (1971).

Moore, Barrett and Brown (1972), using a competitive protein binding technique, measured the progesterone concentration in plasma from the maternal jugular, ovarian and uterine veins and from ^{the} foetal umbilical vein. They reported that the CL made a considerable contribution to the total progesterone produced by the mother through a large part of gestation. The CL was actively secreting progesterone at an estimated rate of 3 to 4 mg/day until about 20 days before parturition, while the placenta secretes relatively little progesterone during the first 2 months of pregnancy, and increases markedly later on. The progesterone levels in the

foetal vein rose as pregnancy progressed but at no stage did they approach those recorded in uterine vein blood.

Peripheral Plasma Progesterone:- Progesterone was measured by several investigators (e.g. Bassett, Oxborrow, Smith and Thorburn, 1968; Fylling, 1970; Stabenfeldt, Drost and Franti, 1972). They reported that following a fertile mating, peripheral plasma progesterone concentrations in the ewe increases at the same rate as in the luteal phase of the normal oestrous cycle. At the period 13 to 26 days after mating, however, there is no decline similar to that seen in the normal oestrous cycle and values in the pregnant ewe remain in the same range as peak luteal phase values for about the first 50 days of gestation, consistent with the view that the CL is the main source of progesterone up to this time. After this time, however, there is a steady increase in the progesterone concentration, peaking 130 to 140 days after mating. The concentration of progesterone in peripheral plasma remained stable only from days 11 to 50 of pregnancy (Bassett et al., 1969). From Day 50 of pregnancy the concentration of progesterone in peripheral plasma rose slowly from 2 ng/ml and reached a concentration of 13 ng/ml on Day 130, when the concentration declined slowly and fell rapidly 1 to 5 days before parturition. Ewes carrying one foetus had a significantly lower level of progesterone in the peripheral plasma than ewes with more than one foetus. In contrast to the above studies, in which a rise in progesterone levels starting about Day 50 of pregnancy, onwards, was reported, Moore et al. (1972), using

the same techniques, reported that progesterone levels remained fairly uniform throughout pregnancy, in peripheral and uterine venous plasma.

Pregnancy Diagnosis using Peripheral Plasma

Progesterone:- The plasma progesterone concentrations in pregnant sheep during the first four weeks of pregnancy are substantially higher than those of non-pregnant ewes from the 16th to the 20th day post-mating. An early diagnosis of pregnancy should therefore be possible on the basis of progesterone concentration in plasma samples obtained at this time. Robertson and Sarda (1971) have reported a correct diagnosis on 31 out of 33 ewes using plasma progesterone concentration levels obtained on the 17th day after mating. It would appear, therefore, that this could prove a useful means of pregnancy diagnosis under some conditions, although it is unlikely that it will be possible to diagnose multiple pregnancies at this early stage. Thorburn et al. (1969) were able to confirm the earlier observation of Short (1961) that the peripheral plasma progesterone concentration could be positively related to the number of CL in the ovary. However, the likelihood of single pregnancies with two CL present in the ovary makes discrimination of single and multiple pregnancies virtually impossible during the period when the plasma progesterone concentration is solely dependent on secretion by the CL. On the other hand, after 60 days of gestation, when placental production of progesterone becomes significant and the peripheral levels begin to increase, diagnosis of multiple pregnancy on the basis of peripheral plasma progesterone concentration may be possible since

Bassett et al. (1969) showed that the mean plasma progesterone concentration in a group of ewes bearing twin lambs was significantly greater than that in ewes with single fetuses throughout the last two thirds of gestation. Whilst these observations indicate that placental progesterone production is proportional to the number of fetuses, the examination of the relationship between the mean plasma progesterone concentration 125 to 135 days after mating, and the weight of lambs at birth shows that, within a group of ewes, the plasma progesterone level, and presumably the placental progesterone secretion rate, is proportional to the weight of conceptus present in the uterus (Bassett and Thorburn, 1973). Recent observations have indicated that a large proportion of twin bearing ewes can be correctly diagnosed on the basis of a single progesterone determination in the period 90 to 120 days of gestation (Gadsby, Heap, Powell and Walters, 1972), although Bassett and Thorburn (1973) indicated that it has not been possible to determine the optimum time differentiation of single from twin bearing ewes.

Recently, Heap, Laing and Walters (1973) provided further confirmation to earlier finding (Laing and Heap, 1971) of the occurrence of progestagens in cow's milk. They reported changes in milk progestagen concentrations during the reproductive cycle compared with those in plasma, and described a simple and rapid radioimmunoassay of milk progestagen, suggesting that this test may form the basis of a method of pregnancy diagnosis.

A3. The Initiation of Parturition

A3.1. The Endocrine Changes at the Onset of Parturition

In the few days before the onset of labour at term in normal sheep several endocrine changes occur that may be related to the mechanism controlling the onset of labour.

1. In detailed studies of peripheral plasma progesterone concentration of the ewe prior to lambing, Bassett et al. (1969) and Fylling (1971) found that there is a significant decrease in plasma progesterone concentration of the ewe during the last week of gestation. The concentration of progesterone in the peripheral plasma falls from a mean value of about 12 ng/ml 7 days before lambing to approximately 1 ng/ml on the day of delivery (Bassett et al., 1969). However, they found a great variability in the pattern, ranging from a slow decline over the last 2 weeks of pregnancy to one instance in which no fall in concentration preceded delivery. Thorburn and Mattner (1971) have also indicated that this decrease reflects a very rapid decline in the concentration of progesterone in the utero-ovarian vein and its secretion by the ovary and placenta, thereby lending support to the theory proposed by Csapo (1961) that the removal of a progesterone 'block' to myometrial activity heralds the onset of parturition.

2. The levels of free plasma oestrogens increased sharply during the last two days of pregnancy (Challis, 1971).

3. In the foetus there is a several-fold increase in the plasma concentration of corticosteroids during the 3 to 4 days before delivery (Bassett and Thorburn, 1969).

4. The foetal adrenal glands double in size between Days 135 and 147 of pregnancy (Comline and Silver, 1961).

5. An additional endocrine event associated with the onset of parturition has been described by Liggins and Grieves (1971), who measured the concentration of prostaglandins in plasma and tissues of sheep before and during premature parturition induced by foetal infusion of ACTH or glucocorticoids. Prostaglandin ($\text{PGF}_{2\alpha}$) was found in uterine vein plasma during labour although it was not detectable before labour, and the concentration in myometrium and maternal cotyledons increased several fold with the advent of labour. It is possible that the peak of circulating oestrogen before parturition could be responsible for stimulating $\text{PGF}_{2\alpha}$ synthesis and so provide the link between foetal corticosteroids and maternal $\text{PGF}_{2\alpha}$.

From the comprehensive studies of Liggins and his co-workers it is now evident that a decline in progesterone level can no longer be regarded as the primary trigger to parturition in the sheep but would appear to be of secondary consequence of increasing activity in the hypothalamus-pituitary-adrenal axis of the foetus (Liggins, 1968, 1969). Elevation of foetal corticosteroids level in immature foetal lambs by administration of either ACTH or a glucocorticoid leads to premature delivery as would be expected if the rise in corticosteroids concentration occurring in normal lambs near term is the physiological mediation of the foetal control of labour. Prolonged pregnancy associated with adrenal hypoplasia induced experimentally by hypophysectomy (Liggins, Kennedy and Holm, 1967; Comline, Silver

and Silver, 1970), pituitary stalk section (Liggins, 1969) or after bilateral adrenalectomy (Drost and Holm, 1968) gives added weight to this concept.

In the goat, Folley and Knaggs (1965) reported a rise in plasma oxytocin to detectable levels in the second stage of labour as the foetus is expelled. They suggested that the role of oxytocin in parturition is probably of a secondary nature and related to the completion of parturition rather than to its initiation. Also Liggins et al. (1972) suggested that it is unlikely that either a fall in plasma and myometrial concentration of progesterone or a rise in circulating oestradiol 17β is the prime determinant of parturition and it remains to be seen whether $\text{PGF}_{2\alpha}$ will fulfil this role.

From the evidence available to them, Davies and Ryan (1972), in a review of comparative endocrinology of gestation, concluded that the timing of parturition is a neuroendocrine function adopted by the foetal hypothalamus and mediated via the pituitary-adrenal system. For the final pathway it is possible that the preparturient diminution in progesterone secretion, probably facilitates parturition by a secondary mechanism through reflex release of oxytocin.

A3.2. Artificial Induction of Parturition

From the above discussion on the initiation of parturition it has been assumed that artificial stimulation of the foetal pituitary-adrenal axis might enable parturition to be induced in farm animals at a specific time. In the later stage of pregnancy, parturition has been successfully

induced by administration of glucocorticoids to ewes (Fylling, 1971; Bosc, 1972), cows (Adams and Wagner, 1969) and goats (Van Rensburg, 1970).

In these studies, dexamethasone infused into the foetus (0.6 to 4 mg/24 hours) induced parturition, while infusion of 4.0 mg/24 hours into the ewe was ineffective. An infusion of 6 mg/24 hours did however result in parturition in 4 days (Adams and Wagner, 1969; Bosc, 1972; Fylling, 1971). Thorburn et al. (1972) reported that a decline in the maternal progesterone level followed infusion of synacthen into the foetus in both sheep and goats. It has also been reported that while 4 mg/24 hours of dexamethasone had no effect on the concentration on blood progesterone in pregnant ewes, 6 mg/24 hours caused a decline in progesterone concentration comparable to that preceding spontaneous parturition (Fylling, 1971). This evidence indicates that corticosteroids can depress progesterone secretion and the declining progesterone level seems to be important for the normal completion of parturition.

The sheep placenta is relatively impermeable to both ACTH and cortisol (Bassett and Thorburn, 1969) and maternally administered corticosteroids cause premature labour only after large doses are given late in pregnancy when placental permeability to cortisol has developed.

A4. The Reproductive Activity after Lambing

A4.1. Lactation Anoestrus

The opinion is widely held that the absence of oestrus in the post-partum ewe is a state of anoestrus due to lactation per se, but the effects on the length of post-partum anoestrus of removing lambs from their mothers at birth, or at various stages during the suckling period have not been well demonstrated and the results are conflicting.

a. Oestrus and Fertility:- The difference in mean post-partum interval between lactating and dry ewes reported by Hafez (1952) was not subjected to a statistical test; the number was small and the variation in interval, for the non-suckling ewes, was wide. On the other hand, Torrell, Clegg, Weir and Cole (1956) concluded that suckling did not influence the incidence of oestrus, or the conception and fertility of ewes during the subsequent breeding season. Miller and Wiggins (1964) studied the occurrence of ovulation in the post-partum ewe in 5 and 6 year old lactating Rambouillet ewes which had lambed in October or November (breeding season). In 12 ewes, laparotomies at the time of the first heat, 20 to 68 days post-partum showed that a silent ovulation had previously occurred in each case. In 40 out of 45 similar ewes a silent ovulation occurred 27 to 84 days after lambing. This was determined by taking smears of cervical mucus at 2 day intervals and confirmed in eight of the ewes by laparotomy. Van Niekerk and Mulder (1965) reported that the early heat periods during post-partum tend to be considerably shorter than usual and if ewes are 'teased' at 12 or even 8

hourly intervals some of the shorter heats may start and end between two consecutive teasings and escape recording. Barker and Wiggins (1964) concluded that lactation, particularly for four months post-partum, was not an important inhibitor of post-partum oestrus, but in ewes that suckled lambs for less than 10 days the post-partum interval to oestrus was 25 days less than in ewes that continued lactating for a longer period. The effect of variation in milk yield between ewes on post-partum intervals to oestrus in ewes suckling 0,1 or 2 lambs as observed by Barker and Wiggins, approached significance, whereas the results of Smith (1964) and Pretorius (1966) showed significant effects of suckling on the post-partum interval to oestrus when the lambs sucked for 2 to 4 months, suggesting that both lactation and intensity of lactation may have an influence on this interval.

Post-partum oestrus has been recorded in sheep (Barker and Wiggins, 1964; Mauleon and Dauzier, 1965; Nel, 1965; Newton and Betts, 1967). However, there is no record of a ewe being successfully mated until 23 days after lambing (Nel, 1965). Nel divided the post-partum period into fortnightly intervals and concluded that between 14 to 28 days after parturition oestrus reappears at a comparatively high rate, but conception is negligible; between 28 to 56 days, conception takes place at about 50% efficiency and only after 56 days is conception normal. Newton (1969) recorded a shorter interval from parturition to conception of 36 to 46 days with Dorset Horn when an effect of litter size on the

interval from lambing to re-mating was apparent. Ewes having twins had a longer post-partum interval than ewes having singles.

For ewes to produce soon after the previous lambing, they must not only be able to show oestrus and produce fertilized ova, but their uteri must also be prepared to provide a proper environment for the developing embryo. Part of this preparation by the uterus is its involution, or return to a prepregnant size and condition to support another pregnancy. Robinson (1959) reported finding in the uterus what appeared to be blood undergoing autolysis. It appeared that material arising from post-partum haemorrhage had been retained through inability to pass the cervix. Similarly, Heap, Allen and Lamming (1963) reported that a uterine washing of lactating sheep six weeks after lambing was contaminated with blood and debris. Heap et al. (1963) also reported that the levels of several chemical constituents of uterine washing in seasonal and lactating anoestrous sheep induced to breed by progesterone-PMS treatment were also different from those of the normal breeding cycle. Thus it appears that unfavourable uterine environment might affect fertilization and embryonic mortality, although later results (Foote, 1968) indicated that the uterus is prepared to support pregnancy by 17 days after lambing.

b. The Effects of Suckling:- To determine the duration of post-partum anoestrus due to lactation Mauléon and Dauzier (1965) observed ewes which had lambed in September or October, in the middle of the breeding season. These

were either dried off between the 2nd and 8th day post-partum, milked, or allowed to suckle; they noted that even among the October lambing ewes, in which the effect of seasonal anoestrus was thought to be minimal, that 18% had only a single heat period before becoming apparently anoestrous.

Hunter and Leishman (1967) compared post-partum intervals to ovulation and oestrus in ewes whose lambs were weaned either at 3 or 20 days post-partum and found no significant difference.

Restall (1971) concluded that the presence of the lamb appeared to block the expression of regular oestrus. However, pituitary activity was evident whether lambs were present with ewes or not, although the mean time of first ovulation was later in suckling ewes. These results were confirmed by Mallampati, Pope and Casida (1971), who reported that the length of post-partum anoestrus was shorter in ewes sucked for only one day as against ewes sucked for 42 days irrespective of the season of the year, and Gould and Whiteman (1973), who reported that post-partum oestrus activity and subsequent reproductive performance in ewes weaned either at 30 or 70 days were higher in the early weaned. A larger percentage of the early weaned ewes mated post-partum to first oestrus and conceived (65 vs. 56% and 58 vs. 38% respectively).

Fletcher (1973), however, concluded that it would not be necessary to wean lambs early in lactation in order to promote the rebreeding of ewes after lambing. Ewes with restricted suckling, or with lambs removed on the day after birth, showed their first post-partum ovulation and oestrus at about the same time as ewes which reared their lambs normally. The

effect of removing lambs on the day after birth was so small that post-partum anoestrus could be regarded as a consequence of gestation and/or parturition and not of subsequent lactation.

c. The Effects of Environmental Factors:- Hunter (1968) in his review concluded that there is, in fact, little good evidence of the nature of lactation anoestrus. He assumed that most of the evidence was based, generally, on observations made following the normal spring lambing of breeds which have short breeding seasons. The majority of such ewes are usually seasonally anoestr~~ous~~ at that time, even if they have not recently been pregnant and not lactating. It therefore seems probable that ewes with inherently longer breeding seasons will generally return to oestrus sooner after lambing than short season breeders, but the return will nevertheless be influenced by the date of lambing. Furthermore it seems that the length of post-partum anoestrous period is likely to be shorter in ewes which lamb nearer the start or peak of the breeding season than in those which lamb later in the season. Cole and Miller (1935) however were of the opinion that the ewe becomes anoestr~~ous~~ after parturition and that the length of anoestrus depends upon the season in which parturition occurs and on the length of the suckling period. Van Niekerk and Mulder (1965) found that there was considerable withinⁱⁿbreed variation in the length of post-partum anoestrus. Breeds differing in the length of post-partum anoestrus have been reported also by Barker and Wiggins

(1964), and Joubert (1962). The latter noted in particular, that cross-breeding both Merinos and Black Head Persians with the Dorset Horn reduced this period in the cross-breeds by about 50% as compared with the parent breeds. Sacker and Trial (1966) found East African Black Headed ewes regularly conceiving 3 to 4 months post-partum while still suckling their lambs. Land (1971) reported the incidence of oestrus in lactating Finnish Landrace, Dorset Horn and their cross Finn-Dorset ewes between 0 and 56 days after spring lambing. Of the animals studied, 100% of Finnish Landrace, 68% of Dorset Horn and 59% of crossbred ewes showed oestrus, and of those mated 77%, 29% and 40% respectively conceived.

Other environmental factors such as nutrition, rather than lactation itself have been suggested to be responsible for post-partum anoestrus. Hunter (1971) indicated that it is difficult to ensure that lactating ewes consume sufficient food to meet their requirements even with the provision of reasonable quantities of supplementary grain. From a series of experiments, Hunter and Lishman (1967); Hunter, Belonje and Van Niekerk (1970); Hunter (1971), Hunter and van Aarde (1973) concluded that if lactating and non-lactating ewes are fed to meet their respective nutritional requirements (NRC suggestion) the length of post-partum anoestrous period will not differ. This contradicts the conclusion that suckling in the cow will delay post-partum oestrous activity independently of nutritional intake (Short, Bellows, Moody and Howland, 1972).

A4.2. The Endocrine Basis of Anoestrus of Lactation

There is evidence that autumn lactating sheep show a silent heat prior to the first full oestrus (Miller and Wiggins, 1964; Mauléon and Dauzier, 1965). It is possible that the hormonal evidence of this silent ovulation may be important in facilitating proper involution of the uterus so that the ewe approaches oestrus with its breeding tract in a condition adequate for sustaining pregnancy as well as for oestrus. Thus it seems that in the post-partum ewe, the recommencement of breeding cycles must await an adequate "build-up" of pituitary gonadotrophins followed by suitable triggering of the releasing mechanism.

a. Follicle Stimulating Hormone (FSH) and LH:-

Mallampati et al. (1971) studied seasonal differences in the FSH and LH concentrations in suckling and non-suckling ewes. FSH concentration in the pituitary glands of ewes sucked for only one day was higher than in ewes sucked for 21 days. The concentration of LH did not differ significantly with either season or suckling. In the bovine, however, Saiduddin et al. (1968) reported that just after calving the pituitary contains more FSH and less LH than 20 to 21 days later. Similar results were obtained from a survey conducted at intervals after calving on the changes in the hormones which may influence post-partum ovarian function from 0.5 to 60 days after calving (Erb, Surve, Callahan, Randal and Garverick, 1971). Plasma LH was significantly lower at 0.5 and 3 days than at 8 and 13 to 60 days after calving. Higher LH values were noted in non-suckling cows in day 7

and 21 post-partum by Short et al. (1972). These were caused by cows being near or at oestrus.

In the sow, Crighton (1967) concluded that lactation inhibits LH synthesis and FSH release. Also, Rothchild (1967) has suggested that, in the rat, suckling may inhibit ovulation by a neural mechanism involving the inhibition of gonadotrophin synthesis as opposed to gonadotrophin release from the pituitary. Also in the rat, follicular quiescence and maintenance of CL during lactation have been attributed to the ability of the suckling stimulus to suppress the secretion of gonadotrophins from pituitary gland on the one hand, and to induce secretion of prolactin on the other (Everett, 1966).

A hypothesis has been put forward (Louw, Erasmus, Mouton and Hansel, 1964) in which it was suggested that the reduction in secretion of gonadotrophic hormones by the lactating ewes may be the result of the greater metabolic load of ewes in this condition and the concomitant increase in secretion of TSH. Other endocrinological mechanisms may be at least partly responsible for post-partum anoestrus; for instance the recent influence of the placenta, and for about a month post-partum, the involution of the uterus and the possible secretion of large amounts of prolactin during this period. Recently Fletcher (1973) reported that ewes which had their lambs removed on the day after birth and were injected intramuscularly with 5 i.u. oxytocin 10 times each day for the first 17 days after parturition, showed their first post-partum ovulation and oestrus significantly earlier

than suckling and non-suckling ewes. This report is contradictory to the inhibitory effects of exogenous oxytocin on luteal function recorded in cattle, (Armstrong and Hansel, 1959) and sheep (Milne, 1963). In cows, Echtermkamp and Hansel (1973) reported that LH concentration in the plasma was low at parturition and remained at low level of 0.5 to 3.0 ng/ml except on the day of oestrus at which time large increases were noted. These LH values agree with those of Arije, Wiltbank and Hopwood (1971) and Edgerton and Hafs (1971) who reported levels of 1.0 to 1.50 ng/ml post-partum with sporadic values up to 3.0 ng/ml.

Pelletier and Thimonier (1973) investigated the effects of lactation on the induced pre-ovulatory LH discharge in anoestrous ewes. The discharge of LH in the lactating ewes was only 65% of that in the non-lactating female; the difference was in both the maximum concentration of LH detected (79 ± 14 vs. 106 ± 9 ng/ml) and in the duration of the discharge (9 hours 50 minutes \pm 50 minutes vs. 12 hours 55 minutes \pm 60 minutes). In agreement with results obtained in rat (Rithchild, 1960) and sow (Crighton and Lamming, 1969), indicating that LH synthesis and release are reduced, these results in the ewe show that pre-ovulatory induced LH release is also reduced during lactation.

b. Prolactin:- Work published by Johke (1969) indicated that, in the cow, ^{the} release of prolactin in response to milking may be related to the stage of lactation. This was investigated further in the goat by Hart (1973), who reported a fall in the prolactin peak levels from 1030 ± 178 to 146 ± 63 ng/ml,

when the average milk yields fell to less than half of their earlier levels. Hart also reported that the stimulus of milking, as well as causing an immediate massive release of prolactin from the anterior pituitary gland, may also initiate a gradually decreasing chronic release of prolactin continuing until the afternoon milking when the process is repeated. Thus the lactating goat is exposed to far higher levels of prolactin than are found in virgin females (47 to 77 ng/ml) or in lactating females which have not been subjected to the milking stimulus for 6 to 7 hours (50 to 100 ng/ml).

In sheep, Davis et al. (1971) noted a decrease from serum prolactin levels ~~from~~ 400 ng/ml at parturition to levels of 100 to 300 ng/ml, during the first 33 days of lactation. Plasma prolactin levels were found to fluctuate between 30 and 60 ng/ml in the non-lactating anoestrous ewe and between 100 to 400 ng/ml during periods of prolactin secretion. Machine milking was associated with a rapid rise in prolactin concentration to levels of 150 ng/ml (Fell, et al., 1972).

The question of a possible relationship between the intensity of suckling and the concentration of pituitary prolactin was investigated in the rat by Tucker, Pope and Sinha (1967), who have shown that increasing the litter size from 2 to 6 pups can cause a rise in the concentration of prolactin in mother's pituitary. These workers attributed this increase to greater synthesis of the hormone. They also presented data to show that, whilst the suckling of 4 pups was sufficient stimulus to deplete the pituitary

prolactin of the mother, the sucking of 2 pups was ineffective. Grosvenor and Mena (1971) also showed that the degree of vigour with which the same number of pups suck may influence the fall in pituitary prolactin of the mother.

A5. Hormonal control of Reproduction

A5.1. The hormonal treatment

Progestagen treatments to control oestrus and ovulation in sheep whether in the breeding season, or during anoestrus are based in trying to simulate the activity of the cyclic sheep's CL, especially its action in producing progesterone. In applying progestagen treatment in anoestrous sheep, the aim is to duplicate those events which normally occur at the start of the natural breeding season, which are the sensitising of receptors in the brain so that the ewe will subsequently respond to ovarian oestrogen by exhibiting oestrus (Robinson, 1959). In most out of season applications of progestagen it is also considered necessary to augment the supply of pituitary gonadotrophins by injecting a follicle stimulating agent at the end of the progestagen treatment. The most readily available gonadotrophin for this purpose is pregnant mare serum (PMS).

Hormones can be used to control reproduction in at least the following areas: (1) to synchronize breeding during the breeding season: (2) to increase the incidence of multiple births during the breeding season; (3) to induce fertile mating (oestrus, ovulation and preparation of uterus) during anoestrus. The main advantages of synchronizing breeding are to control the length of the lambing period and the number of ewes lambing during a particular period, which would help accommodate available labour, facilities and feed, and to facilitate special breeding programs, including artificial insemination. Some management problems, however,

would be expected where large groups of ewes are synchronized for breeding.

Under some management programmes it may be advantageous to increase the proportion of multiple births. This might be the case where ewes are bred late in the season and ovulation rate is normally lower due to ^{the} influence of season. It might also be the case where ewes are under an intensive management programme. Injections of 400-1000 i.u. PMS near the end of the oestrous cycle have consistently increased the number of lambs born per ewe. Increases of 35 to 75% have been recorded (Neville and Neathery, 1964).

The day of the oestrous cycle that PMS is injected appears to influence its response. Neville (1966) reported birth rates of 1.00, 1.44 and 1.78 lambs per ewe when injections were given on days 12, 13 and 14, respectively. Hulet and Foote (1967) compared time of PMS injection at first and second oestrus after progesterone treatment in a limited number of ewes and found that the best response was obtained on the first day and 15 to 17 days following the end of progesterone treatment. Responses were measured in terms of both ovulation rate and number of embryos at 25 days.

The available data indicate that the number of lambs born from breeding during the regular breeding season can be increased by treatment with PMS and that the day of treatment is important.

Successful induction of fertile matings during the non-breeding season with the use of hormones depends on recognition and adequate control of several environmental

factors. If the ewe is to conceive from natural mating, she must experience concurrent oestrus and ovulation. In addition, the uterus must be properly prepared for pregnancy. Progesterone plays an important role in preparation of the uterus for pregnancy. In addition, progesterone also acts to influence ^{the} production and/or release of hormones from the ewe's own pituitary gland which facilitates production of oestrus and ovulation. In this same regard PMS might act on the pituitary gland to cause release of ovulating hormones (gonadotrophins) (Quinn and Zarrow, 1964) as well as to act more directly on the ovary to cause ovulation.

The majority of hormone treatments used to produce out-of-season breeding in ewes have involved only progestagen and PMS. The schedule has been to pretreat with progestagen for 12-16 days followed by PMS between 0 and 48 hours later to cause oestrus and ovulation. Hulet and Foote (1967) reported comparisons of dry and lactating ewes fed different types of progestagens for different lengths of time in relation to two PMS injections. There was no difference in response due to type of progestagen used. The percent of ewes lambing and lambing rate was highest in the groups receiving longer progestagen pre-treatment.

The Progestagens Employed

The response to a progestagen treatment depends on the particular progestagen employed, the dose level and the particular procedure used. Although a great range of progestagen agents have become available over the past

decade, mainly for use in human fertility control; very few of these are considered appropriate for use in controlled breeding in sheep. This was evident from the very comprehensive screening work conducted by Australian workers on progestagens (see monograph by Robinson, 1967). The Australian workers showed that only compounds with characteristics identical to progesterone, in having a very short biological half life, are suitable. Efforts were concentrated largely on one such compound - Cronolone (G.D. Searle). In the various tests conducted with this steroid, the physiological action of cronolone did not appear to deviate from that of progesterone itself, except in being at least 20 times more potent. It is this high potency which enables an effective dose (30 mg) to be incorporated with ease into sponge pessaries. Recent results indicated that the addition of progesterone (400 mg) to the cronolone pessary could lead to further improvements in the lambing outcome (Gordon, 1971a). Treatment is for a 12 day period. Oestrus and ovulation usually occur between the 2nd and the 5th day following the end of treatment.

A5.2. Fertility at Control Breeding

The development of the method for administering progestagen by polyurethane sponge pessaries opened up new possibilities for sheep production control under commercial farming conditions. However, there is much evidence to show that the 'synchromate' treated ewes were not achieving maximum fertility (Gordon, 1971b). In the autumn breeding season, extensive trials/ 'synchromate' pessaries among more
with

than 1000 cycling sheep showed conception rates averaging somewhat less than 60%, even though the mating response was in the order of 95% (Wishart, 1967).

Consideration of the initial dose of cronolone used in impregnation and its degree of dispersion within the pessary led Robinson (1968) to suggest that the progestagen administration procedures and doses previously employed in controlling the breeding may have been too low to duplicate all the normal functions of the sheep's CL. There is much evidence to show that conception rate in progestagen treated sheep can vary according to the time interval between sponge withdrawal and the onset of heat (Gordon, 1971 b); the earlier sheep came in oestrus after treatment, the poorer the conception rate.

Another factor to be considered in the progestagen treated sheep, is that oestrus and ovulation might occur when the breeding tract is still very much influenced by the residual effects of progestagen, operating in the tract for some time after withdrawal of the hormone from the circulation (Robinson, 1960). In this situation sperm transport may be adversely affected (Quinlivan and Robinson, 1969). There may be a failure in the establishment of an adequate sperm population (Allison and Robinson, 1970), or there may be an alteration of sperm capacitation, and in the rate at which sperms are phagocytosed by leucocytes in the tract, as has been suggested in cattle (Lauderdale and Ericsson, 1970). This could mean that with larger than normal sperm doses, sufficient sperm will still reach

the upper oviducts to ensure fertilisation. This could be one factor in explaining how ram management procedures in which ewes are bred to each of several rams, or are 'hand bred' to rams of high fertility, may result in conception rates of 80% (Jennings and Crowley, 1972; McClelland and Quirke, 1971).

The possibility that pretreatment with oestrogen might further enhance the induction of oestrus and ovulation and subsequent fertility in anoestrous ewes receiving progestagen and PMS has been investigated (Foote, 1968). In these trials the incidence of both oestrus and ovulation and the subsequent proportion of pregnant ewes was consistently higher in animals receiving oestrogen pre-treatment.

Fertility returns to the pretreatment level at the second post-treatment oestrus (Foote and Waite, 1965). Also once ewes have been synchronized, they usually remain well synchronized through at least the first three post-treatment oestrous periods (Foote and Waite, 1965). This allows the scheduling of treatments so that the ewes can be bred at the desired date at the second rather than the first post-treatment oestrus. Under this management, the level of fertility is as high as in untreated ewes.

Early studies (Gordon, 1958; Allen and Lamming, 1960) indicated that in induced breeding activity of anoestrous sheep, conception rate was higher in treated maiden sheep than in treated lactating sheep. However, Gordon (1963) reported that treated ewes which were nursing

lambs 3 to 4 months old at mating, conceived as readily as non-lactating sheep. Also Foote (1968) concluded that there is no clear evidence of a detrimental effect of lactation on fertility in controlled breeding.

B. THE NUTRITIONAL ASPECTS OF REPRODUCTION

B1. The Effects of Nutrition on the Reproductive Cycle

Of the environmental and hormonal interrelationship which are intimately associated with the very complex process of reproduction, nutrition should be regarded as one of the most prominent factors. Reproduction in sheep is primarily controlled by the endocrine activity of the pituitary gland and the gonads, although the role of other endocrine glands is also indirectly important (Asdell, 1957). In a review, Folley (1949) pointed out possible ways in which nutritional conditions could influence reproduction by affecting 1) the anterior pituitary; affecting the maturation, shedding and implantation of ova; occurrence and timing of oestrus and the maintenance of pregnancy. 2) the nervous system; influencing neural control of the hypophysis. 3) the metabolism of sex hormones; influencing levels in the body through changes in the rates of production or inactivation. 4) the responsiveness of target organs to sex hormones. 5) the ingestion of sex hormones or substances specifically affecting the activity of endocrine glands.

It has been widely recognised that undernutrition of immature animals delays sexual development in both the structure and function of the gonads. There is also a general view that the level of nutrition and the condition of the ewe at lambing time influences the ability of the ewes to conceive again promptly. These views have not always been supported by detailed studies but are considered of sufficient importance to merit further research.

Bl.1. Oestrus

A high plane of nutrition when compared to a low plane has not always been found to stimulate sexual activity and lead to a higher ovulation rate. The effect of sub-maintenance on the onset of the breeding season was studied by Hafez (1952) who concluded that nutrition has no more than a minor effect on the breeding season and the occurrence of oestrus in sheep. Wallace (1963) fed ewes from weaning to mating on high, medium and low planes of nutrition and also found that different feeding regimes had little effect on the time of onset of the breeding season. However, Roux (1936) reported that Merino ewes on high planes of nutrition underwent a mean number of 11.2 oestrous cycles during the breeding season, while only 7.2 cycles were observed in the breeding season of those on a low plane of nutrition. Van Niekerk and Mulder (1965) also suggested the difference in feeding levels may be the main source of the variation reported in the literature for the post-partum interval to oestrus in a particular breed of sheep even when lambing occurs at the same time of the year in each instance. Hunter (1961) and Smith (1964) have demonstrated that the level of oestrous activity in Merino ewes during the expected peaks is also influenced by the plane of nutrition during the preceding season. This was confirmed by Vosloo, Hunter and Carstens (1969) who subjected Dorper ewes to either high, medium or low feeding levels throughout the last 6 weeks of gestation and during the subsequent lactation period of 100 days. Analysis of variance of the results revealed that the

high feeding treatment significantly reduced the post-partum interval to ovulation.

Bl.2. Ovulation

McKenzie and Terrill (1937) reported that the ovulation rate in low plane ewes was lower than for comparable animals on a high plane of nutrition, and El-Sheikh, Hulet, Pope and Casida (1955) found that a high level of feeding in ewes resulted in significantly more ovulations and larger follicles than those receiving lower intakes. In a literature review, Reid (1960) concluded that greater numbers of eggs on the average were produced by the more liberal feeding levels in swine and sheep, but the fertilisation of the egg was not greatly affected. In sheep Casida (1963) suggested that the percentage of multiple ovulations was greater in the groups receiving grain in addition to hay; the difference due to the feeding ranged from 20 to 62% in different trials. Similarly Howland, Kirkpatrick, Pope and Casida (1966) also reported a higher ovulation rate in high plane ewes, than in low plane (1.71 versus 1.42). Recently Lamond, Hill, Godley, Kennedy and Gaddy (1973) reported that on a low energy level 12 of 16 ewes were found in oestrus, 15 ova were produced and 9 were fertilised. In the high energy group, however, 14 of 16 ewes were observed in oestrus, 23 ova were produced of which 8 were fertilised. Thus it seems that the influence of body weight at mating on lambing performance appears to be largely a reflection of differences in ovulation rate (Edey,

1968), though, at low body weight, embryonic mortality may also be involved (Edey, 1970).

From the works reviewed above, it seems that there is a favourable effect of a high level of nutrition on ovulation rate, although, Allen and Lamming (1961) reported that ovulation continued to occur after a loss of 28% of initial body weight which indicates a reduced effect of nutrition on reproduction in the ewe than in the rat where similar losses of live weight are associated with dietary anoestrus.

Bl.3. Fertility

The conception rate and lambing rate were reported to be affected by nutrition (Girou, Theriez, Molenat and Auguer, 1971). In their study, ewes were fed different levels of concentrates for 4 weeks prior to insemination and for the following 3 weeks. The ewes that were well fed initially had the highest conception rate and lambing percentage, whilst those on low feeding following insemination had the highest embryonic mortality. Of those ewes on high feeding levels before and after, 80% subsequently lambed while only 51% of ewes receiving no supplementary feeding produced lambs. Cumming (1971) reported that a rising plane of nutrition did not result in a significantly higher ovulation rate than a falling one, but resulted in a significant increase in both the percentage of ewes lambing and the number of lambs born per ewe mated or per ewe lambing. Similarly, Gunn, Doney and Russel (1972) reported that a good body condition



at mating had a significant positive effect on both ovulation and lambing rates and had an apparent negative effect on embryo mortality up to day 26 ± 2 after mating.

Studies by Foote et al. (1959), Edey (1969) and Hill, Godly and Lamond (1969) showed that high levels of intakes of nutrients before and during a breeding period do not always achieve the expected increase in lambs born. This was confirmed by Lamond et al. (1973) who observed that prolonged high intakes reduced fertilisation rates because of poor quality ova and inadequate uterine function. They concluded that the deleterious effects of over-nutrition and under-nutrition appeared to have been mediated through the same general mechanism affecting both ova and the genital tract simultaneously.

Bl.4. Flushing Effects

The effect of a rising plane of nutrition for a short term before and during mating produces the well known and long established phenomenon of flushing. There is no definition of when flushing should start, for how long it should continue nor what type of feed should be used. The response to flushing as measured by ovulation rate was however reported to depend on the duration of the flushing period (Allen and Lamming, 1961; Lamond and Bindon, 1969). The effects of nutritional stimulation has been studied in sheep and swine (Casida, 1963) and the minimal time for enhancement of ovulation rate when flushing with grain showed that the contemporary feed level appears to be of minor

importance in the sheep, as opposed to swine. Longer term concentrate feeding, on the other hand, showed advantages over flushing.

The physiological basis of the flushing stimulus is not clearly understood. Allen and Lamming (1961) suggested that nutrition might affect ovarian activity by altering: 1) the rate of secretion and/or release of gonadotrophins from the pituitary gland, 2) tissue reactivity to the action of these hormones, or, 3) the metabolism of this hormone. But it is still uncertain which of these factors causes the ovarian response. More follicles undergoing the immediate pre-ovulatory enlargements in the flushed ewes may have been due to either a greater gonadotrophin release at oestrus or less regression of follicles very late in the cycle, or both.

Whether or not increased ovulatory response on high starch rations was a result of more energy or a unique action of starch requires further study. Sheep normally have blood glucose concentration within the range 50 to 70 mg % and they utilise Volatile Fatty Acids (VFA) as a major source of energy. Since brain tissue cannot utilise VFA as an energy source (McClymont and Setchell, 1956) their blood glucose becomes a limiting factor for cellular activity in the brain. Bellows, Pope, Meyer, Chapman and Casida (1963) suggested that some of the increase in the carcass weight of flushed ewes may be due to an increase in protein material and postulated that flushing in ewes is associated with increased rates of protein synthesis, which in turn makes a major contribution to the glucose requirement by

gluconeogenesis (Lindsay, 1959). In grain-fed ewes, the greater propionate available from starch fermentation may allow the plasma glucose to be maintained at an elevated level as well as to provide a sparing effect on protein. Thus the elevated blood glucose level could provide a stimulus to the hypothalamic centres controlling gonadotrophin leading to greater hormone production and subsequent ovarian activity. The possibility of pure energy sources added to a good growing ration was explored in swine by Zimmerman, Spies, Self and Casida (1960), through the feeding of a glucose to the basal ration. This was successful in increasing the number of CL by 2.1 (11.8) as compared to the basal ration (9.7); lard and corn oil have also been successful in increasing the ovulation rate. Sodium propionate or glucose had no significant effect when given for a short time only. It seems, therefore, that flushing can be due to high level of energy whether supplied as grain (Howland, et al., 1966) or lard or glucose (Zimmerman, et al., 1960) or corn oil (Kirkpatrick, Howland, First and Casida, 1967).

B2. The Effects of Nutrition on the Endocrine Control.

Mulinus and Pomerantz (1940) suggested that the effects of nutrition are mediated through the pituitary gland which in turn affects the function of the gonads. These workers noted that there is a similarity between the effects of under-nutrition and of hypophysectomy and adopted the term "pseudo-hypophysectomy" to cover the endocrinology

syndrome of undernutrition. Evidence about the effect of nutrition on the structure and activity of the pituitary with special reference to the anterior pituitary were reviewed by Ershoff (1952) and Leatham (1958). In separate reviews dealing with nutritional effects on hormone production, Leatham (1966) and Lamming (1966) advanced the pseudo-hypophysectomy theory as a possible explanation for ovarian hypofunction seen under low levels of nutrition. According to this theory, the ^{effects of} nutritional difference in animals undergoing oestrous cycles lies in the amount ~~of, or the~~ length of time, that gonadotrophins are released. However, the possibility exists that the nutritional effect is upon the number of follicles susceptible to gonadotrophin stimulation cannot be eliminated. To identify the response of a target organ to gonadotrophin stimulation from an effect on the secretion, estimations of hormonal levels in the blood should be carried out.

B2.1. Gonadotrophins Secretion

Darlow (1942) compared the effects of maintenance and production rations on ewes slaughtered at six different stages of the oestrous cycle. The gross gonadotrophic activity of the pituitary gland was tested on immature female rats but no difference in the gonadotrophic activity of statistical significance was found, nor was there significant inter-action of stage and feed levels. The pituitary glands from the ewes on the gaining ration were, however, heavier than the glands of those on the maintenance ration so that the total potency was presumably greater. Although little attempt has been made to study the endocrine mechanisms

involved in nutritional anoestrus in farm species, evidence in the rat suggests that under-nutrition leads to impaired release of gonadotrophin from the anterior pituitary gland (Piacsek and Meites, 1967). They reported that in rats which were fed 50% of normal diet no reduction in the pituitary concentration of FSH was observed, but that of LH was reduced when compared to ad-lib fed control. There was also a reduction in the hypothalamic content of LH-RH in the underfed rats which is consistent with the reduced levels of plasma LH observed by Howland (1972). Howland (1971) also reported reduced ovarian weight and function due to deficiency in basal LH level. Cooper (1970) working with pigs demonstrated that sows on a low level of feed had approximately four times as much LH left in their pituitaries when slaughtered at oestrus as compared to pigs given extra feeds on the first day of acceptance of the boar. It thus appeared that the extra feed stimulated a greater release of LH already present in the pituitary, although subsequent work has not supported the earlier held view.

Some work has been reported for sheep with regard to nutritional effects on pituitary gonadotrophic content. Allen and Lamming (1961) found no significant difference in pituitary concentration of total gonadotrophins on different feeding regimes even though there were significant differences in ovulation rate. Bellows et al. (1963) reported that the LH and FSH contents were greater in the flushed sheep, than in those receiving a grain supplement, due to the greater anterior pituitary weight and not the concentration per mg of

tissue. The blood glucose level was also greater in the flushed sheep and the authors suggested this might stimulate the hypothalamus directly, leading subsequently to greater ovarian activity as seen by the significant increase in ovulation rate. Similarly, increased pituitary weight by grain feeding was also reported by Howland et al. (1966). The pituitary LH concentration was higher in grain fed ewes at all stages of the cycle, but no direct effect of feed on FSH concentration was apparent. A parallelism between total gonadotrophin potency and follicular development at all stages was interpreted to mean that there was greater hormone production in grain fed ewes.

In cattle, Hill, Lamond, Dicky and Niswender (1970) reported no change in basal plasma LH in beef heifers receiving 85% of the National Research Council (NRC) recommendation (NRC, 1964). In contrast, Gombe and Hansel (1973) reported progressive increases in plasma LH both in basal levels and peaks during 3 oestrous cycles in energy restricted heifers. These changes were not in the direction that would have been expected from the pseudo-hypophysectomy theory, since plasma LH in the energy restricted animals was significantly elevated rather than depressed. Thus the decreased ovarian function associated with a low energy intake cannot be explained on the basis of reduced plasma LH as appears to be the case in underfed rats. Gombe and Hansel (1973) further suggested that the first effect is a reduced ability of the ovarian tissue to respond to LH.

Correlation between feeding and the blood level of

prolactin have been reported by Schams and Karg (1970) who showed that there was a fall in the circulating level of prolactin when food was withheld from lactating cows, heifers and bulls for 24 hours. The possible role of prolactin in carbohydrate metabolism has been considered and its anabolic effects have been tested in cattle. McAtee and Trenkle (1971) found that infusion of arginine monohydrochloride into fasted and fed pre-pubescent heifers caused a prolonged increase in the circulating levels of prolactin, whereas infusion of glucose had no effect.

B2.2. Progesterone.

Donaldson, Bassett and Thorburn (1970) noted small, statistically significant rises in plasma progesterone followed by significant decline in progesterone values, in cattle restricted to 25% of the total food intake of controls. Reduced plasma progesterone within 5 days in cattle receiving 85% of maintenance requirement for energy and protein was reported by Hill et al. (1970). Measurements of progesterone were also made by Gombe and Hansel (1973) in undernourished heifers which were maintained for 3 oestrous cycles on either 100% or 62% of Total Digestible Nutrients (TDN) allowances. During the first cycle, plasma progesterone was slightly higher in the low caloric group, but became progressively lower in the subsequent cycles. Both total progesterone and progesterone concentration in CL taken on the 10th day of the third cycle were lower in the low TDN heifers than in the control group.

Hill et al. (1970) reported that there was no relation between plasma progesterone and the size of the CL. They concluded that it is possible that the effect on plasma progesterone values may not be due entirely to altered secretion rates by the CL. Gombe and Hansel (1973), however, suggested that the first effect of restricted energy intake is at some stage ~~on~~ steroidogenesis within the CL causing a reduction in plasma progesterone.

In addition to these reports in ^{the} bovine, Lamond, Caddy and Kennedy (1972) reported a significantly higher progesterone concentration in a group of ewes which were fed a maintenance ration than in 2 other groups which were on either ad libitum feeding or on pasture. Higher progesterone concentration in early pregnancy of under-nourished ewes was reported by Cummings, Mole, Obst, Blockey, Winfield and Gooding (1971), who stated that the progesterone levels on 25% of maintenance ration were significantly higher than those of 100% maintenance. The same results were reported by Donaldson et al. (1970) for undernourished cows in mid and late pregnancy.

The cause of the rise in plasma progesterone during under-nutrition is as yet unexplained but as progesterone is essential for the maintenance of pregnancy Cumming et al. (1971) postulated that the increase in plasma progesterone might be due to decreased metabolic clearance rate, mobilisation of stores or ^{an} increased rate of secretion.

B3. The Nutritional Requirements of the Ewe.

Probably the most common aspects of nutritional deficiency of sheep are due to a limitation of energy intake.

This may result from lack of sufficient feed or from the low net energy availability of the feed consumed. Poor quality roughages are not only poorly digested but are also consumed in smaller quantities. Although an energy shortage may be complicated by other deficiencies, such as protein, minerals, or vitamins, inadequate energy intake will usually result in a slowing or cessation of growth, loss of weight, reproductive failure and increased mortality. It is accepted that internal parasite infection is more severe in sheep suffering from malnutrition because of their lowered resistance. Such factors as air temperature, wool length, exercise and gestation are known to affect sheep's feed requirement and feed utilisation and have been studied by Armstrong, Blaxter, Graham and Wainman (1959), Lambourne (1961), Coop (1962), Langlands, Corbett, McDonald and Puller (1963) and Coop and Clarke (1967).

Acute experiments with exteriorised foetuses of ruminants (Barcroft, 1946) have led to the belief that the foetus is largely dependent on glucose for its energy supply (Reid, 1960a). Twin foetuses near term, weighing e.g. 6 kg could require as much as 900 Kcal/day (Graham, 1964) whereas the energy content of the glucogenic nutrients (propionic acid and protein) derived from a normal diet (e.g. 1 kg/day of food which is 70% digestible) would be no more than 1000-1500 Kcal/day. Thus, if normal food intakes were reduced by 10-40%, the glucose which could be derived from the diet would all be required by the foetus. Although foetal growth rate would probably decrease in such

circumstances (Graham, 1964), the occurrence of pregnancy toxaemia in undernourished pregnant ewes (Reid, 1960a) indicates that the foetal supply may be sustained to the detriment of the ewe. It has been claimed by Reid (1960a) that gluconeogenesis from protein cannot affect the maintenance of homeostasis in undernourished pregnant ewes because amino acids are required in large amounts for foetal growth. However, some results (Graham, 1964) suggest that foetal nitrogen requirements amount to only 1 or 2 g/day, which is not large in relation to the wastage of 5 to 10g/day in an average maintenance ration (Graham, 1964).

B3.1. Metabolic Changes in Undernourished Animals

Levels of nutrient intake are associated with variation in the metabolism of fat in the body, especially the mobilisation of depot fat during low intakes. Related to this process are changes in levels of non-esterified fatty acids (FFA), ketone bodies (Aceto-acetate, β -hydroxybutyrate, and acetone), and glucose in the blood. FFA are released in the blood plasma when adipose tissue is mobilised to supply metabolic needs of the animal, primarily the need for energy. Although the quantity of FFA in the blood of ruminants is small, it is an important factor in caloric homeostasis of the body. Physiological regulation of the release of FFA from adipose tissue has not been well defined in ruminants, but the mechanisms of FFA metabolism appears to be similar to those in man and non-ruminants, as reviewed by Frederickson and Gordon (1958a & b).

Both hormonal status and nervous control are known to affect the release of FFA by adipose tissue (Engel and White, 1960), and this process plays a central role in the process of caloric homeostasis proposed by Frederickson and Gordon, 1958b. Increased glucose utilisation by adipose tissue depresses the release of FFA and accelerates the rate of fatty acids and neutral fat synthesis (Bally, Cahill, Le Boeuf and Renald, 1960). FFA are oxidised in the liver to produce acetyl CoA and ketone bodies (acetoacetic acid and β -hydroxybutyric acid). The latter are normally further catabolised to CO_2 and water in extrahepatic tissues but they accumulate in the blood in conditions of hypo-glycaemia or reduced glucose utilisation. In the adequately nourished animals the concentration of plasma ketone bodies expressed as acetone is less than 3 mg/100 ml.

The extent to which an undernourished ewe is catabolising body fat may be measured by the concentration of FFA in the plasma (Annison, 1960). In ewes fed once daily, at, or above, a maintenance intake, there is a diurnal variation in the concentration of plasma FFA from a level of 100 to 200 μ equiv/litre 2 to 3 hours after feeding, when the ewe is laying down surplus nutrients as fat, to 500 to 600 μ equiv/litre during the latter part of the 24 hour period when the ewe is mobilising fat. The plasma FFA concentration increases progressively with starvation or continued undernourishment until maximum levels between 1500 and 2500 μ equiv/litre are reached (Annison, 1960). Carbohydrate content of the ration fed can affect the level of FFA in the blood.

Annison (1960) and Reid and Hinks (1962a) found that, in sheep, the decrease in plasma FFA after feeding was greatest with rations having the highest content of carbohydrate.

Plasma FFA are most useful as an index to the degree of undernourishment over a range of approximately 500 to 1200 μ equiv/litre. Plasma FFA concentration as a criterion of undernourishment has been discussed by Reid and Hinks (1962a) and Patterson (1963). The rate of increase of plasma ketones as hypoglycaemia develops in the undernourished animals is not as great in the early stages as that of FFA (Reid and Hinks, 1962b), therefore this parameter is consequently a less sensitive index of a moderate degree of undernourishment than FFA concentration, but it is a more useful index in cases of severe undernourishment where the FFA levels are approaching, or have reached, their maximum.

B3.2 The Assessment of the Adequacy of Feeding.

The criteria used for establishing optimum nutrient intakes for ruminants are not always adequate. In a review Thomson and Aitken (1959) adopted absolute gain or loss of live weight of the ewe as an indirect criterion of plane of nutrition in an attempt to correlate the results of different studies of ewe nutrition. Weight changes have some relevance to farming practice and comparison with other experiments can often be made only on the basis of weight changes owing to the range of conditions under which they were carried out. Robinson and Forbes (1968) also suggested that a net body weight change of the ewe over the period is a more accurate

index of treatment response. Treacher (1970) suggested that nutritional treatments during the last third of pregnancy in the ewe can influence birth weight of lambs particularly twin lambs. Russel, Doney and Reid (1967a) have reasoned, however, that although such a result may often be the case, lamb birth weight is the least satisfactory criterion by which to assess the adequacy of a particular level of energy intake. In the majority of experiments on the nutrition of the pregnant ewe, groups of ewes are fed at different levels of nutrition, the object being to establish uniform nutritional states within a group and to look for differences between groups in subsequent production as measured by lamb birth weight. However by supplying equal amounts to all ewes in any one group, a variety of nutritional states are produced depending on foetal weight, which is itself the principle criterion by which the effects of a treatment are to be judged.

The method used to define nutritional treatments in many investigations of undernutrition have been criticised by Russel, et al. (1967a). They indicated the need for techniques that permit all ewes on a treatment to be maintained in an equal nutritional state relative to the size of foetal burden. Annison (1960) was the first to suggest that the level of plasma FFA could be a useful indicator of nutrient status of the ruminant, particularly as to carbohydrate utilisation. Results of studies with pregnant ewes (Russel et al., 1967a) support this suggestion. Russel et al. (1967a) suggested that the usefulness of plasma FFA

and blood ketone levels as indicators of nutritional status may vary with the degree of undernourishment, and when treatments are defined as particular levels of FFA or of ketones in the blood plasma, the level of undernutrition in the ewe can be directly related to the size of the foetal burden of the individual ewe, irrespective of her weight or body condition. They concluded that this procedure for controlling feed intake to a prescribed degree of undernutrition was effective and applicable over a range from moderate to severe undernourishment. From information collected in this study it was also possible to estimate the additional requirements of the ewe for energy in late pregnancy (Russel et al., 1967b), and the effect of nutritional level during pregnancy on the milk production of the ewe as reflected in lamb growth rate (Peart, 1967). Sykes and Field (1972) using this technique were successful in maintaining uniform levels of FFA within group - 1100 to 1300 μ equiv/litre - which are typical values in severely undernourished sheep, but were not as successful as Russel et al. (1967a) in equating feed intake with foetal demand. They also found considerable variation in relative changes of FFA and ketone bodies between sampling times, the results being a tendency to overestimate the increments for those animals with small foetuses and underestimate those for ewes with large foetal burdens. Sykes and Field (1972) could not maintain FFA below 600 μ equiv/litre even though the ewes were on ad libitum intakes and maintained net body weight during pregnancy, whereas values below 250 μ equiv/litre are

easily maintained in non-pregnant sheep. It seems therefore of interest to examine the interrelationship between the hormonal changes occurring during pregnancy and their influence of the plasma metabolites.

B3.3. Recommended Nutritional Standards

Following the advances in the development of metabolisable energy (ME) as a system to describe both the energy value of a given feed and the animal's energy requirement (ARC's "Nutrient requirements of farm livestock: No. 2. Ruminants") the ME system has been adopted throughout this thesis.

ME is a measure of the energy which feeds contain, rather than the production which they can support in the animal. It is determined experimentally as the gross energy of the feed, less the losses of energy in faeces, urine and methane. When the way in which ME is utilised for particular forms of production (e.g. growth or lactation) is known, then feed requirements for those purposes can be expressed directly in ME terms. Other energy terms are: Digestible organic matter (DOM), 1 g of which is equal to 3.75 Kcal ME according to Blaxter and Wainman (1964), and Total digestible nutrient (TDN). A conversion factor of 1 lb TDN = 1.72 Mcal ME was suggested by Garrett, Meyer and Lofgreen (1959).

a. Maintenance Requirements:- Armstrong et al. (1959) Lambourne and Reardon (1963), Coop (1962) and Langlands et al., (1963) have reviewed the energy requirements of sheep. Maintenance requirements have also been calculated from the

regression of live weight change per unit weight on intake per unit weight by Doney and Russel (1968). Whichever form of experimental design or analysis is used it appears that the various maintenance requirements for non-pregnant sheep do not differ widely and are of a similar order to those indicated in "The nutrient requirements of farm livestock" (ARC, 1965) - 8 to 9 g/DOM/Kg/Ewe or 33 Kcal/ME/Kg/Ewe.

Thomson and Aitken (1959), and Phillipson (1959) indicated that the maintenance requirement of non-pregnant ewes for protein were 3.6 g Digestible Crude Protein (DCP)/Kg W^{0.73}, or about 60 g/day for 50 Kg ewe. This is similar to the USA NRC (1964) recommendation of approximately 54 g DCP/50 Kg ewe. There is, however, evidence that the maintenance requirement for protein may be much lower than these levels suggest. Two estimates of maintenance requirements obtained by Robinson and Forbes (1968) were 3.53 ± 0.71 and 2.84 ± 0.38 g apparently digested N per ewe per day. These are approximately one-third of the allowance by the (USA) NRC but similar to the values obtained by Elliott and Topps (1964).

b. Non-Lactating and first 15 Weeks Gestation:- In view of the relatively small size of the foetus 45 to 55 days pre-partum (Wallace, 1948 and Langland and Sutherland, 1968) the additional energy requirements of the ewe attributed to pregnancy at this early stage are expected to be low. For example the NRC recommendations exceed only slightly those necessary for maintenance during this period. Coop and Clark (1967) have substantially confirmed these recommendations with large scale field trials involving 3500 ewes. In these

studies 2 to 7 weeks pregnant ewes were fed either at approximately maintenance level or at approximately half maintenance for a further 5 to 8 week period. They concluded that restricted nutrition at the level and time applied had no effect on reproductive performances of ewes and therefore the practice of restriction in early pregnancy to conserve feed for late pregnancy is a sound policy.

c. Last 6 Weeks of Gestation:- It is possible that the energy requirements set forth for the last 6 weeks of gestation should be higher in order to meet the needs of the growing foetus and associated tissues, to allow proper development of mammary tissue, and to maintain the ewe's body weight and condition. The feeding of the ewe in late pregnancy has received considerable attention over the years, but because of the variability of body conditions, there is still very little precise information available on the nutritional requirements of the ewe during that time. The ARC (1965) stated the nutritional requirements of a pregnant ewe could not be clearly defined. The majority of published reports have been concerned with the effects of specific diet or feed supplement in pregnancy on subsequent performance as measured in terms of lamb birth weight, early lamb growth and other indices of parturition. Recently attempts have been made to relate these criteria to the intakes of energy or other nutrients supplied by the wide variety of diets used.

Russel et al. (1967a) have used the concentration of FFA to adjust the feed intake of pregnant ewes throughout gestation to maintain a predetermined range of nutritional states. From these data, Russel et al. (1967b) calculated

an estimate of the additional requirements for energy in late pregnancy of 100 g DOM/Kg/24 hours relative to early pregnancy which is equivalent to a daily energy requirement of 375 Kcal ME/Kg foetus. Assuming a maternal maintenance requirement of 8 to 9 g DOM/Kg (Coop, 1962; Lambourne and Reardon, 1963; and Langlands et al., 1963), it can be calculated that the feed intake required to prevent undernourishment in late pregnancy in a 50 Kg ewe with an average sized single foetus is of the order of 900 g DOM/day. A similar ewe with average sized twin foetuses would require a further 350 g DOM/day. This estimate is approximately twice that calculated by Langlands and Sutherland (1968) using a comparative slaughter technique, and also higher than the estimate of the energy required for the promotion of what was considered an adequate level of ewe live weight gain in late gestation (Robinson and Forbes, 1968). In an attempt to explain the diversity of these estimates of energy requirements of the pregnant ewe, an experiment was carried out by Robinson, Fraser and Bennett (1971) in which ewes were offered different amounts of feed intake during gestation. From the relationship between FFA concentration, energy intake, lamb birth weight and ewe body weight, estimates of the energy requirements of the pregnant ewe were predicted. The additional ME intake above maternal maintenance required to prevent elevation of plasma FFA concentrations above a predetermined basal level, 5 days pre-partum was calculated as 335 Kcal/g lamb birth weight, which is very similar to those estimates given by Russel et al. (1967b).

Investigations into the effects of protein intake during the second half of gestation on lamb birth weight and ewe milk yield during early lactation have given variable results. There is also some variation between experiment results and intake levels recommended in practice. The reviews of Thomson and Aitken (1959) and Phillipson (1959) concluded that 0.25 lb DCP per day should be adequate for a 140 lb ewe during the last 6 weeks of pregnancy. The NRC (1964) recommended 0.20 lb for the same ewe weight, but ARC (1965) found it difficult to make firm recommendations. They indicated a requirement of 0.30 lb DCP/day for a 150 lb ewe. There is, however, evidence that the requirement for protein may be much lower than those levels suggested. Robinson and Forbes (1968) have shown that pregnant ewes will remain in positive N-balance during late gestation on a daily intake of DCP as low as 70 g. McClelland and Forbes (1971) also reported that in-lamb ewes that were given 38, 58 and 82 g DCP daily (during the last 6 weeks of gestation) at a constant energy intake of 600 Kcal/ME per head, there were no significant differences between treatments in live weight gain of the ewes, lamb birth weight or ewe net body weight change (the difference between live body weight gain over the experimental period and live weight loss at lambing).

Russel et al. (1967b), Davies, Johnston and Ross (1971), Robinson et al. (1971) and Sykes and Field (1972) recommended a low/high pattern of feeding during late pregnancy which allows a progressive increase in intakes of energy and protein. This low to high pattern of feeding corresponds more closely to the demands of the developing foetus which

was shown by Wallace (1948) to increase three-fold in weight between 16 to 20 weeks of gestation. It also ensures that the ewe is on an increasing plane of nutrition during late pregnancy, a condition regarded as important in the prevention of pregnancy toxaemia (Forbes and Singleton, 1964). Elsley, Bathurst, Bracewell, Cunningham, Dent, Dodsworth, MacPherson and Walker (1971), however, doubted the necessity to adopt this changing pattern in feed intake during pregnancy because of an increasing efficiency of utilisation of nutrients, in particular protein, found with pregnant sows (Elsley, Anderson, McDonald, MacPherson and Smart, 1966) and with pregnant ewes (Robinson and Forbes, 1968). They claim that there is no advantage in a low/high type of feeding sows in terms of mean birth weight of the piglets and concluded that the pattern by which feed is distributed during pregnancy is far less important than the total feed given during gestation. In a recent paper McClelland and Forbes (1973) found no significant effect of the mean body weight loss between mating and post-partum by either energy intakes or pattern of intake during the last 6 weeks of gestation. Under practical conditions, therefore, a constant feed intake throughout pregnancy seems to be the easiest method of feeding farm animals.

d. Lactation:- The requirements of lactating ewes for differing levels of milk production have been stated more clearly than in pregnancy (ARC, 1965; USA NRC, 1964). It has been assumed that in addition to the basic requirements for maintenance a daily allowance in the ration of the average lactating ewe suckling single lamb amounts to

0.75 Kg TDN or 2.7 Mcal ME (NRC, 1964). Ewes suckling twins have higher yields and therefore greater nutritional requirements than ewes suckling a single lamb. According to a Meat and Livestock Commission report (MLC, 1972) a 68 Kg ewe suckling twin lambs and maintaining her body weight will require 6 Mcal ME/day and 340 g DCP/day. However, Hunter (1971) assumed that it is not easy to ensure that lactating ewes consume sufficient food to meet their requirements which are beyond their capacity even with the provision of reasonable quantities of supplementary grain, and so lactating ewes will inevitably lose weight.

SUMMARY OF LITERATURE AND AIMS OF THE STUDY

In the comprehensive literature reviewed on reproduction in ewes some aspects of the normal cycle are well documented, but there is, however, a lack of definite information on aspects of post-partum sexual activity and the relationships between level and mechanisms by which nutrition affects reproductive activity in the ewe.

Such information is essential if the aim of an accelerated lambing system is to obtain two lamb crops a year. Such a system requires animals with an extended breeding season and shortened post-partum and lactation anoestrus. Thus the ewes will resume sexual activity and be able to conceive soon after the previous lambing.

Nutritional aspects

The ability of any ewe to express her reproductive potential depends on adequate nutrition. Her needs are highly seasonal and at present they are rather ill-defined. Clearly the pregnant animal has different requirements from those of the ewe in lactation and the needs of each may vary according to the number of lambs carried or suckled. Failure to meet requirements at any one time may greatly alter the requirements during a subsequent period.

The nutritional plane may affect the time lapse from birth to first breeding. Nutrition could be one of the major contributory factors to when ewe lambs can breed and lamb as yearlings and continue to breed in consecutive years, thus increasing their productivity on a lifetime basis. Ewes

usually receive extra feed before and during the mating period, i.e. 'flushing', but although there is an abundance of research on 'flushing' its influence is still not resolved. Some works report an increase while others report no increase in ovulation or lambing rate. In some trials there was no correlation between weight gain during the flushing period and the lambing performance of the ewes, as there were ewes under some conditions at pasture that produced good response in lambing performance, while others in as good conditions in drylot gave poor lambing performance. It is likely that the response to 'flushing' is dependent upon a range of related genetic, environmental and management factors. Such factors include handling, age, breed, time of year, previous history together with feed level, and type of feed. It is certain that some of these factors vary from one trial to another and thus results must be carefully interpreted.

In practice very little attention has been paid to the nutritional status of the multiparous ewe during pregnancy, and ewes with multiple fetuses have often been treated similarly to those with one, which can be related to a high neonatal loss of lambs. It is obvious that a valid assessment of the usefulness of larger litters or any techniques employed to produce them, cannot be made unless the nutritional status of the ewe is adequate. At the present, there is little information on what an adequate level of nutrition is in relation to normal litter size, quite apart from foetal burdens in excess of the normal. It cannot be assumed that ad libitum feeding will automatically dispose

of these problems. Whether or not a pregnant ewe with any particular foetal burden, can ingest sufficient nutrients, is likely to depend on the nature and quality of the food offered and not merely on the quantity available. With increasing foetal number it may be anticipated that the quality of the food will become more critical. It has been suggested that the abdominal space is restricted during the second half of pregnancy by the presence of the foetuses and related maternal tissues, and ewes carrying triplets consumed less food towards the end of pregnancy than ewes carrying twins or a single. Much more information is required and this can only be obtained by experimentation with large litters.

Similar arguments apply to the results of lamb growth and mortality after birth. The chances of survival and performance of lambs cannot be fairly assessed unless the ewes were adequately fed during pregnancy. Also there is a question of milk output and whether it is satisfactory to leave a large litter with the ewe. It has also been suggested that the level of feeding during pregnancy may affect the development of udder tissue. The feeding level during lactation is by far the most important influence on milk yield, and lactating ewes may voluntarily consume more than the dry ewes although lactating ewes commonly lose weight during the first 4 to 6 weeks of lactation. Their ability to do so without a reduction in milk yield, must be related to their weight and condition immediately after lambing, and this in turn may reflect nutrition during pregnancy. It is

certainly true that the stress on the ewe is far greater during lactation than it is during pregnancy, and it is therefore likely that the frequency of lambing that can be achieved may be dependent of the ewe's status during lactation. Thus the relationship between nutrition, milk yield and weight loss should be better understood in order to predict the outcome with any confidence.

From the literature survey, it seems that there is no reliable method of assessing the nutritional status of the ewe, except under certain restricted conditions. Size of sheep or body condition should be carefully defined before use, as barren ewes that are kept year after year will quite naturally be amongst the largest sheep in the flock. Body weight may also be a poor measure in itself as two sheep of the same weight may represent extremes in body condition. Body condition, or degree of fat cover, may also be of limited value because it is commonly a subjective judgment. Composition of gain also complicates interpretation as an increase of weight may be fat, lean, water or combinations, e.g. weight gain during pregnancy may reflect a higher percentage of water.

Adequate ewe nutrition during pregnancy depends upon knowing how many foetuses each ewe has, but there is not yet a simple, reliable test for pregnancy in sheep that can be used early enough. Electrocardiography and the use of X-rays are not effective until heart-beats or bones are sufficiently developed. For early pregnancy testing, laparotomy is the only practical procedure available at the present time. It is known that if the ewe has conceived the plasma progesterone concentration remains elevated at the end of the oestrous

cycle and continuously rises until the end of gestation. It seems therefore of interest to assess the use of plasma progesterone concentration to determine pregnancy and multiple foetuses in a flock situation.

The length of gestation is very significant in a six-month lambing interval system. Based on the classical work of Liggins and his co-workers in which they demonstrated the role of the foetal pituitary in the initiation of parturition and the particular importance of the hypothalamic-pituitary-adrenal axis, it would be possible to induce and synchronise a normal parturition in sheep. The synchronisation of parturition will make the whole lambing period shorter and will have great advantages as it will affect the practicability of the intensive breeding programmes.

Reproductive activity during post-partum period

The earlier literature on the factors affecting re-breeding during the post-partum period has been reviewed in detail by Hunter (1968). Briefly, it was concluded that the resumption of sexual activity following lambing at different times of the year is influenced by seasonal factors and that the widely held opinion that sexual activity is also affected by lactation may have arisen partly from the fact that lactation in many ewes commonly coincides with their anoestrous period. There is little and conflicting evidence concerning the existence of a lactation anoestrus, which can only be resolved by further investigation. A firm recommendation regarding the need for early weaning to promote the occurrence of post-partum heat cannot be made

until well planned experiments have been carried out in which ewes that lamb and lactate during their natural breeding season have been studied; otherwise the effect is complicated by seasonal anoestrus.

Most progress has been made in recent years with the elucidation of the role of progesterone in facilitating the induction of co-incident oestrus and ovulation and with the development of extremely potent progestagens. However, it is clear that several problems remain unresolved, the most important being that of sub-fertility at the treatment oestrus. The cause of failure is still undetermined, but defects in sperm transport and survival, exposure of sperm to abnormal leucocyte activity in the uterus, or a failure of sperm to become capacitated may be involved. Sub-fertility is more acute in progesterone-treated anoestrous ewes than in cycling animals, presumably because the progesterone is acting on a tract which is relatively undeveloped in comparison with that in sheep which have passed through one or more normal ovarian cycles. Oestrogen treatment in conjunction with a progestagen has been found to aggravate the sub-fertility. Much more needs to be known about hormonal relationships at oestrus, especially between oestrogen and progesterone. In attempting to facilitate conception in the post-partum ewe there is also a need to know much more about factors influencing involution of the uterus, and especially how involution can be influenced by management and hormonal procedures. The nutritional status of the ewe at the time of treatment may be very important and this in turn may be influenced by both pre-lambing and post-lambing feeding. By analogy with what

is known in cattle, conception rate in the early months after parturition may be poorest in ewes that are losing weight rapidly at the time of breeding (Wiltbank, 1970).

The part played by nutrition in the resumption of oestrus, ovulation and fertility in post-partum ewes has received little attention. Hunter and Van Aarde (1973) pointed out that the plane of nutrition during lactation may have an important influence on the resumption of sexual activity. It is therefore possible that by good feeding levels, short heats, heats of low intensity or the silent ovulations which have been reported to occur during the post-partum period, could be converted to normal heats, but further research is necessary to establish this clearly.

The endocrine control of the normal oestrous cycle in the ewe is relatively well documented but little is known about its activity during the post-partum period, and how the endocrinological control is affected by nutritional stimulation or deprivation; it is not known whether the animals change the secretory pattern of pituitary gonadotrophins or whether the reproductive function is affected by the degree of sensitivity of the target organs, the availability of metabolites, the metabolism of the hormones or other factors. Nutrition may also influence the neural centres concerned with gonadotrophin release. With the development of the extremely sensitive protein binding assay and radioimmuno-assay techniques, it is possible to undertake much more comprehensive studies of the endocrinological control in the processes of reproduction and to link up physiological studies with research into nutrition.

The use of hormone treatments to promote early re-breeding may be necessary if lambing at 6 month intervals is the objective, but as has been shown the results of such treatments are at present unpredictable. Investigations in the direction of understanding the basic endocrine status of the post-partum ewe will facilitate the development of a rational programme of feeding, management and hormone therapy designed to reduce to a minimum the post-partum interval to effective rebreeding. The body condition and the nutritional status of the milking ewe at the time of breeding are of great importance in obtaining an acceptable conception rate. It can therefore be concluded that there is a great need to link up controlled breeding studies with nutritional studies; certainly this is an area in which there are many gaps in our knowledge.

In accordance with the points outlined above the following aims were selected for the present study.

1. The adequate diet for the pregnant ewe, especially through the final weeks before parturition, will be studied by measuring the performance of Finn-Dorset first cross ewes under various planes of nutrition. The objectives will be to determine the optimal nutritional regime and to measure the response to it in terms of ewe and lamb performance and the subsequent breeding performance during the first 6 weeks after lambing. Also a criterion will be established which will accurately measure the optimum nutrient intakes by analysing blood samples to determine the

rate at which the ewe is drawing on body reserves to meet the deficit between requirements and the feed supplied.

2. So far it is only possible to control oestrus and ovulation and, by implication, fertilisation. No method has yet been applied to control the natural variation in gestation length nor to synchronise or space out parturition within the 24 hours of the day. To take full advantage of controlled breeding, synchronisation of lambing will be carried out by a single injection of a glucocorticoid into the ewe.
3. The effect of lactation on post-partum anoestrus during the breeding season will be studied when the effects of seasonal and dietary deficiency factors will be eliminated as far as possible.
4. The effects of undernutrition during the pre- and post-partum periods on the reproductive activity in non-suckling ewes will be investigated.
5. A detailed study of blood hormone levels (LH, prolactin and progesterone) will be carried out during late pregnancy, induced parturition and the first 6 weeks post-partum.

MATERIAL AND METHODS

Animals

The ewes for these experiments were part of a young flock of first cross (F_1) Finnish Landrace x Dorset Horn (Finn-Dorset) established for the purpose of accelerated lambing studies, the ewes chosen for the experiment were of approximately uniform body condition equivalent to grade 2.5 to 3 on the scoring system described by Russel, Doney and Gunn (1969) averaging 65 Kg live weight and 3 years of age.

The Breed

Claims have been made that the Dorset Horn will show heat during the major part of the year (Newton and Betts, 1967). Ewes and rams of the Finnish Landrace breed were imported to Britain in 1962 (Donald and Read, 1967) with a view to crossing them with existing British breeds including the Dorset Horn. In its own country the Finnish Landrace population is large and long established. The breed is reported to have a mean litter size of 2.5 to 3.0 lambs (Maijala, 1966) and the conventional lambing occurs during the first half of the year (January-June with a few cases in December, July or August).

Finnish Landrace x Dorset Horn (Finn-Dorset) combining a high incidence of multiple births of the Finn with the prolonged breeding season of the Dorset Horn, it was reported to have a high reproductive activity in which both sexes are involved (Land, 1970a,b,c). Land (1971) also reported that the high proportion of this cross displayed oestrus and became pregnant during lactation with no deleterious effect on the number of lambs subsequently born

(Land and McClelland, 1971).

The present productivity of these ewes is sufficiently high to indicate their suitability for studies of intensive sheep husbandry system.

Nutrition

The ewes were on pasture during the spring and summer months, subsequently returning indoors for lambing and lactation at the end of October. Indoors the ewes were fed hay and concentrate or Complete Ruminant Diet (CRD) according to the experimental design.

The ewes were offered concentrates or CRD at 0800 and the chopped hay at 1600 hours. Feed residues were collected daily and bulked over a period of one week. Weekly feed samples were taken for chemical analysis, the mean proximate analyses of the concentrates mixture, hay and the CRD used in the experiments are given in Table 1.

Feeding Scale:- The feed intakes were based on the energy content of the diet used. The ewes were allocated to a particular ration according to ewe live weight measured on two consecutive days immediately before allocation to treatment groups. The basal scale was 33 Kcal ME/Kg ewe live weight/day calculated to meet the maternal requirements for maintenance. During the last 6 weeks of gestation the ewes were given an additional allowance of 365 Kcal ME/Kg foetus/day, on the predicted birth weight assumed for this breed of 3.5, 6.5 and 8.2 Kg for single, twin and triplets respectively (Land and McClelland, 1971). In all experiments the number of lambs carried by the ewe was diagnosed by X-ray on Day 90 of gestation.

TABLE 1Composition of diets, nutrients and digestibility

<u>Constituent (%)</u>	<u>Complete ruminant diet (CRD)</u>	<u>Concentrate</u>
Barley straw	40.00	-
Ext. groundnut meal	7.00	-
Ext. Soya bean meal	-	10.00
Ground barley	12.50	82.00
Wheat feed	23.25	-
Molasses	10.00	5.00
Urea	1.00	-
Tallow	1.25	-
NaCl	1.50	} 3.00
Dical. Phosphate	1.50	
Sodium Bicarbonate	2.00	

<u>Additives</u>	<u>gms/ton</u>
'Adivitamix' AD ₃ E*	200
Cobalt sulphate	17
Ferrous sulphate	36
Manganese sulphate	18
Potassium Iodide	2
Zinc Oxide	20

* declared composition: 50.000 i.u. Vit. A, 10.000 i.u. Vit.D, and 52.5 i.u. Vit. E per gram.

Mean Analysis (%)

	H ₂ O	C.P.	EE	Fibre	NFE	Ash	DCP(%)	Mcal	ME/Kg
CRD	15.9	14.8	2.5	18.3	40.0	8.5	9.6	1.79	
Concen- trates	12.8	12.6	1.3	4.3	63.9	5.1	10.3	2.62	
Hay	21.5	11.0	na	37.9	na	na	5.2	1.77	

Digestibility**(%)

	<u>Digestibility of dry matter</u>	<u>Digestible O.M. in D.M.</u>	<u>DCP</u>
CRD	62.20	58.60	65.0

** based on data of 6 wethers fed maintenance level.

Housing

The flock was situated at Woodhouselee Steading of the School of Agriculture Farms. The farm is situated at approximately 200 m above sea level, at latitude 56° and longitude $3^{\circ}50''$ west. The ewes were kept on pasture throughout the spring and summer months until the end of October, when they were brought indoors and penned individually on slatted floors or on sawdust. Lactating ewes were penned in groups which allowed access to supplementary "creep" feed for the lambs.

Animal Care.

The ewes were shorn in early summer prior to mating in July. Routine regular examination and treatment of feet was undertaken, particularly before housing, with frequent bathing of feet in 6% formalin solution. The ewes were dosed with an anti-helminthic three times during each grazing season and also about 2 weeks after housing. A multi-purpose clostridial vaccine was injected prior to each lambing.

Experimental Methods

Live weights were regularly recorded once a week before morning feeding.

Bleeding: Routine blood samples were obtained before feeding from the jugular vein by the use of heparinised containers under vacuum. Immediately after withdrawal the samples were centrifuged and the separated plasma stored at -15°C pending analysis.

Synchronisation of Lambing: An attempt was made to synchronise lambing by the use of a single intramuscular injection of 16 mg dexamethasone (Berck Pharmaceutical Ltd.) on 141st day of gestation between 20.00 and 21.00 hours. This is discussed more fully in Chapter 2.

After parturition the ewes were weighed, a blood sample obtained within 8 hours after delivery and the lambing data recorded.

Rearing of Lambs: According to experimental design, the lambs were allowed continual access to their dams, for either 24 or 56 days, or weaned 24 hours after lambing and artificially reared on reconstituted milk substitute. The lambs were also given access ad libitum to a high energy intensive fat lamb pellet (creep) from the first week until 12 weeks of age.

Observation of Oestrus: Spontaneous oestrus occurring during the first 3 weeks post-partum was checked with vesectomised rams twice daily at 09.00 and 16.00 hours.

Synchronisation of Oestrus: Intravaginal sponges treated with 30 mg 'Cronolone' (G.D. Searle) and 400 mg progesterone were inserted for 12 days as described by Gordon (1971a).

Laparoscopy: The ovaries were examined with a laparoscope under general anaesthesia (Nembutal, Abbott), in the third week post-partum to check for spontaneous ovulation and in the middle of the synchronised cycle at 6 weeks post-partum. In each case the number of CL on each ovary were counted.

Mating: The ewes were penned with rams known to be fertile on two occasions during the synchronized oestrous cycle, at 40 and 52 hours after onset of oestrus. The ewes were observed for at least one service on each occasion before removing the rams.

Chemical Analysis

Protein: The crude protein of the diets was determined by the macro-Kjeldahl method (official methods of Analysis, 1960).

Energy: The digestible energy was determined by bomb calorimetry of feed and of faeces. Digestible energy values were transformed to metabolisable energy values by the use of corrections for methane energy (Blaxter and Clapperton, 1965) and urine energy (Blaxter, Clapperton and Martin, 1966).

Blood Glucose (mg/100 ml): The glucose levels were determined by an automatic technique described by Gutteridge (1968) using apparatus developed by Technicon.

In the following analysis a detailed description is provided since the methods used differed slightly from those reported elsewhere.

Non-Esterified Fatty Acids in Plasma (FFA): The method used was closely related to that described by Patterson (1963) to estimate plasma FFA concentrations. The detailed procedure is described below:-

Into a 30 ml stoppered test tube was placed 1 ml of plasma to be assayed, and to it was added 10 ml extraction mixture (iso-propanol; n-heptane; 1.0 N-HCl = 40:10:1 (v/v) prepared from analytical grade reagents). The initial

extraction was carried out by vigorous mixing for 30 seconds. After leaving for 5 minutes, 4 ml distilled water and 6 ml heptane were added and the fatty acids redistributed to the upper heptane layer by shaking for 60 seconds. (approximately 82.5% of the FFA fraction was extracted this way.) The upper layer separated rapidly and completely on standing. After 30 minutes there was no difficulty in removing a 5 ml aliquot of the heptane layer which evaporated to dryness in a 25 ml conical flask under nitrogen on a warm hot-plate.

Control extractions were carried out at the same time, 1 ml of water replacing the plasma samples in the above procedure. Replicate standards were also prepared following the control procedure, but using tubes in which 1 ml of a 1 m-molar heptane solution of recrystallised palmitic acid had first been evaporated. Thus pure palmitic acid and plasma FFA were extracted at the same time and under identical conditions.

The residues obtained from blank, standard and plasma extracts were dissolved in 2.5 ml warm 95% ethanol (redistilled from calcium oxide, and titrated with approximately 0.01 N-NaOH using 0.5 ml 0.02% ethanolic Nile Blue solution as indicator (in a stream of nitrogen). A Conway micro-burette was convenient for these titrations. The blue to red end-point was observed to span the addition of less than 2 μ l 0.01 N-NaOH.

The concentration of FFA in plasma was computed from the ratio of the sample to standard titrations (allowance being made for blank titrations) and expressed as μ equiv/litre

calculated as palmitate.

Evaporation of the heptane included in the method excluded volatile fatty acids which are present in ruminant blood at appreciable levels and the ketone bodies likely to be present in ketonaemic plasma samples. It was considered that the interference from ketone bodies would therefore be negligible.

Reproducibility within and between assays was usually comparable to that claimed by Patterson (1963). In a series of duplicate FFA estimations made on samples at levels ranging from 100 to 600 μ equiv/litre it was found that 95% of the pairs of estimations differed by 40 μ equiv/litre or less. The titration values for control samples (water) were 13.6 ± 0.87 , coefficient of variation = 12.1% and for the standard samples (palmitic acid) 116.0 ± 3.23 , coefficient of variation = 10.0%, ($n = 15$). The recovery of palmitic acid, taken as the major typical non-esterified fatty acid (Dole, 1956) was $96 \pm 2\%$ when added to plasma.

The Determination of Ketone Bodies: The quantitative determination of ketone bodies in blood was based on the method described by Reid (1960b). The method is dependent on the conversion of all ketone bodies to acetone, distillation of acetone and its subsequent colorimetric determination by reaction with ethanolic salicylic aldehyde to form dihydroxy dibenzene acetone in alkaline solution.

In the present experiment, the total ketone bodies were determined in one single distillation, assuming that the ratio of acetone + acetoacetic to β -hydroxybutyric acid

is of 1 to 2, the maximum error of this estimate is $\pm 5\%$ if the true ratio lies between 1 to 1 and 1 to 5 (Reid, 1960b).

In the single distillation method described in detail by Reid (1960b), a 5 ml sample filtrate (Somogyi, 1952) was placed in the distilling tube with 3 ml of 18 N sulphuric acid and heating was started and continued until collection of distillate had begun. Potassium dichromate solution (5 ml) was then introduced from the dropping funnel and heating was continued until 5 ml of distillate had been collected in 5 minutes. Colour development was carried out by adding 4 ml of 10 N sodium hydroxide and 2 ml colour reagent solution - ethanolic salicylic aldehyde. The tubes were shaken vigorously and placed in a water bath at 55°C for 20 minutes. After that the tubes were set aside to cool at room temperature for 1 hour and the colour developed was measured against a reagent blank solution, prepared at the same time in 5 ml distilled water, with a spectrophotometer at 520 m μ , using a Vitatron, Digital concentration Photometer.

Standards run with each series of determinations included triplicate of undistilled and distilled acetone standards (0.5 ml and 5 ml per 100 ml respectively) and β -hydroxybutyric acid solution which had been through the entire procedure.

Recovery of acetone from acetoacetic (distilled acetone) varied from 83.0 to 90.2%, coefficient of variation = 10.66%. Recovery of acetone from β -hydroxybutyric was 66.0 to 70.0%, coefficient of variation = 5.93%. The maximum

additional error likely to be introduced by the assumption of an 85% recovery of acetone from acetoacetic acid is less than 0.1 mg per 100 ml (Reid, 1960b).

Luteinising Hormone Assay:

Materials.

Antiserum - An equine antiserum to bovine LH raised in mares immunised with NIH-LH-B₃ by Dr. R. Snook (Iowa, USA) was used in the assay.

Label - Purified ovine LH of biological potency of 1.6 relative to NIH-LH-S17 was used for radioiodination (Carr and Land, 1974).

Standard - NIH-LH-S17 preparation was used for the assay standard.

Solid-Phase Radioimmunoassay: LH was determined by a solid-phase technique using antibody-coated tubes (Catt and Tregear, 1967) and the procedure was similar to that described by Goding et al. (1969).

In the present assay, polystyrene tubes (15 x 90 mm) coated with 0.8 ml of a 1:2500 dilution of antiserum to bovine LH in 0.05 M bicarbonate buffer, pH 9.6. After standing for 3 hours at room temperature, the diluted antiserum was aspirated and the tubes washed twice in 0.15 M NaCl and once with a diluent solution consisting of 0.2% BSA in 0.15 M NaCl and 0.01 M phosphate buffer pH 7.4 (BSA diluent). By this means, the plastic tubes were coated internally with a fixed quantity of antibody to bovine LH.

To perform the assay each sample of sheep plasma was diluted with BSA, and 3 half ml aliquots of 5%, 10% and 20%

plasma were incubated in the anti-LH coated tubes at 37°C. At the same time ovine LH standards of 0 to 8 ng/ml were incubated in triplicate in BSA diluent in identically coated tubes at 37°C. After 18 to 24 hours 50 µl of BSA diluent of ^{125}I -LH was added to each of the standard and sample tubes, and incubation was continued for a further 18 to 24 hours. The contents of each tube were then aspirated and the tubes were washed twice with tap water and counted in an automatic gamma spectrometer (Wallac).

The standard curve and the LH concentration in the plasma samples were calculated using a computer program derived from that of Rodbard and Lewold (1970). The programme gives estimated potency and 95% confidence intervals for each sample. Accuracy and precision of the assay were checked by repeated assay of a pooled plasma sample. Specificity of the method was investigated by testing other pituitary hormones for cross reaction (Carr and Land, 1974). In this anti-bovine LH ^{125}I ovine LH assay system cross reaction with TSH was higher than the LH contamination in the TSH preparation used (NIH-TSH-S6). FSH and GH cross reacted at the level of the reported contamination, but no cross reaction was detected with prolactin at levels as high as 60 ng/ml.

Double Antibody Assay: Plasma samples with low LH concentrations were assayed with a double antibody technique as described by Carr and Land (1974).

In the assay, half ml aliquots of standards (0.1 to 4.0 ng/ml), control (4%, 2% and 1%) in triplicates, and unknown (10%, 5% and 2%) diluted with BSA (2%), were added to polystyrene tubes (LP_3) followed by 200 µl of a 1:400000

dilution of the first anti-serum. The tubes were mixed (Whirlimix), covered and incubated at 4°C for 3 to 4 days.

After the incubation period, 50 µl of BSA diluent of ^{125}I -LH was added to each tube and incubation was continued for a further 3 to 4 days. 200 µl of second anti-serum (Rabbit anti-horse gamma globulin, diluted to 1/60 with BSA diluent containing 9.01 M EDTA and 1/1000 horse serum) was then added to each tube followed by a further incubation overnight.

After the last incubation 1 ml of BSA diluent was added to each tube before centrifugation at 2500 r.p.m. for 30 minutes. Immediately after centrifugation the supernatant was aspirated off and the tubes were inverted over tissue. The tubes were counted and the concentrations calculated as described above.

Progesterone Assay: The plasma progesterone was determined by a radioimmunoassay technique adapted from the method of Hotchkiss, Atkinson and Knobil (1971).

Reagents and Materials

Antiserum - was raised in an 18 month old castrated male British Saanen goat, and was kindly supplied by Dr. B.J.A. Furr (ICI). The antiserum was diluted to 1:4000 in buffer (pH 7.0) consisting of 0.01 M-sodium phosphate, 0.1% NaCl and 0.1% gelatin (PBS). The diluent antibody was stored at 4°C.

Reagent grade n-Hexane, ethanol and dioxane were used after additional purification.

^3H progesterone - was diluted in ethanol and stored at -20°C. An aliquot of the tritiated progesterone solution

was evaporated to dryness under a stream of nitrogen and rediluted (1:90) in PBS 0.1% gelatin before use in the radioimmunoassay. The antibody and tritiated progesterone dilutions were selected empirically to give 40 to 50% binding of labelled progesterone in the control assay tubes containing no unlabelled steroid.

0.25% Norit A in phosphate-buffered saline provided a charcoal suspension which absorbed 98% of the tritiated progesterone in the absence of antibody. This suspension was stored and utilised at 4°C.

The extraction of the steroid from distilled water or sheep plasma was carried out in 15 ml glass-stoppered conical centrifuge tubes, and the radioimmunoassay was performed in 12 x 75 mm glass tubes which had been rinsed with distilled water and ethanol and dried before use.

The liquid scintillation counting medium contained 0.7% PPO (2,5-diphenyloxazole) and 0.03% dimethyl POPOP [1,4-bis-2(4-methyl 5-phenyloxazolyl)-benzene] dissolved in dioxane.

Extraction Procedure of Progesterone

Ethanollic solutions containing 250 pg of ^3H progesterone (2500 cpm) were evaporated to dryness in extraction tubes and redissolved in 100 μl of plasma from post-partum ewes. The tubes were mixed and allowed to stand for 15 minutes at room temperature before extraction. Analysis of these samples provided an estimate of procedural losses.

100 μl aliquots of distilled water or sheep plasma with or without added progesterone were extracted with 1.5 ml n-hexane with hand shaking for 20 seconds and with

whirlmix for another 20 seconds. The n-hexane supernatant was transferred to glass tubes and evaporated to dryness under a stream of nitrogen.

Standard Curve

Aliquots of standard solutions of progesterone corresponding to 20, 50, 100, 200, 300 and 500 pg were added to triplicate glass tubes from which 1.5 ml of n-hexane had previously been evaporated, and dried under a stream of nitrogen.

Radioimmunoassay

After the preparation of the standards and experimental samples, 100 μ l of the antibody solution and 100 μ l of tritiated progesterone (6000cpm) were added to the tubes, shaken briefly and incubated for 16 to 20 hours at 4°C. After incubation, the separation of the antibody-bound and free steroid was performed at 4°C by the addition of 1.0 ml of the charcoal suspension to each tube. After mixing the tubes were allowed to stand for 20 minutes before centrifugation at 1200 x g for 10 minutes at 4°C. The supernatants containing the antibody-bound steroid were decanted into scintillation vials and counted to 10,000 counts in 10 ml of scintillation fluid in a Packard model 3320 liquid scintillation spectrometer. The progesterone content of each experimental sample was determined from the standard curve and corrected for procedural losses. The data are expressed as ng progesterone/ml plasma.

Losses During Extraction

The losses were determined from the recoveries of 250 pg tritiated progesterone added to 30 samples, averaged

$76.53 \pm 1.43\%$, coefficient of variation - 5.6%. In this study the recovery of 250 pg ^3H progesterone was used routinely in assessing procedural losses in the assay.

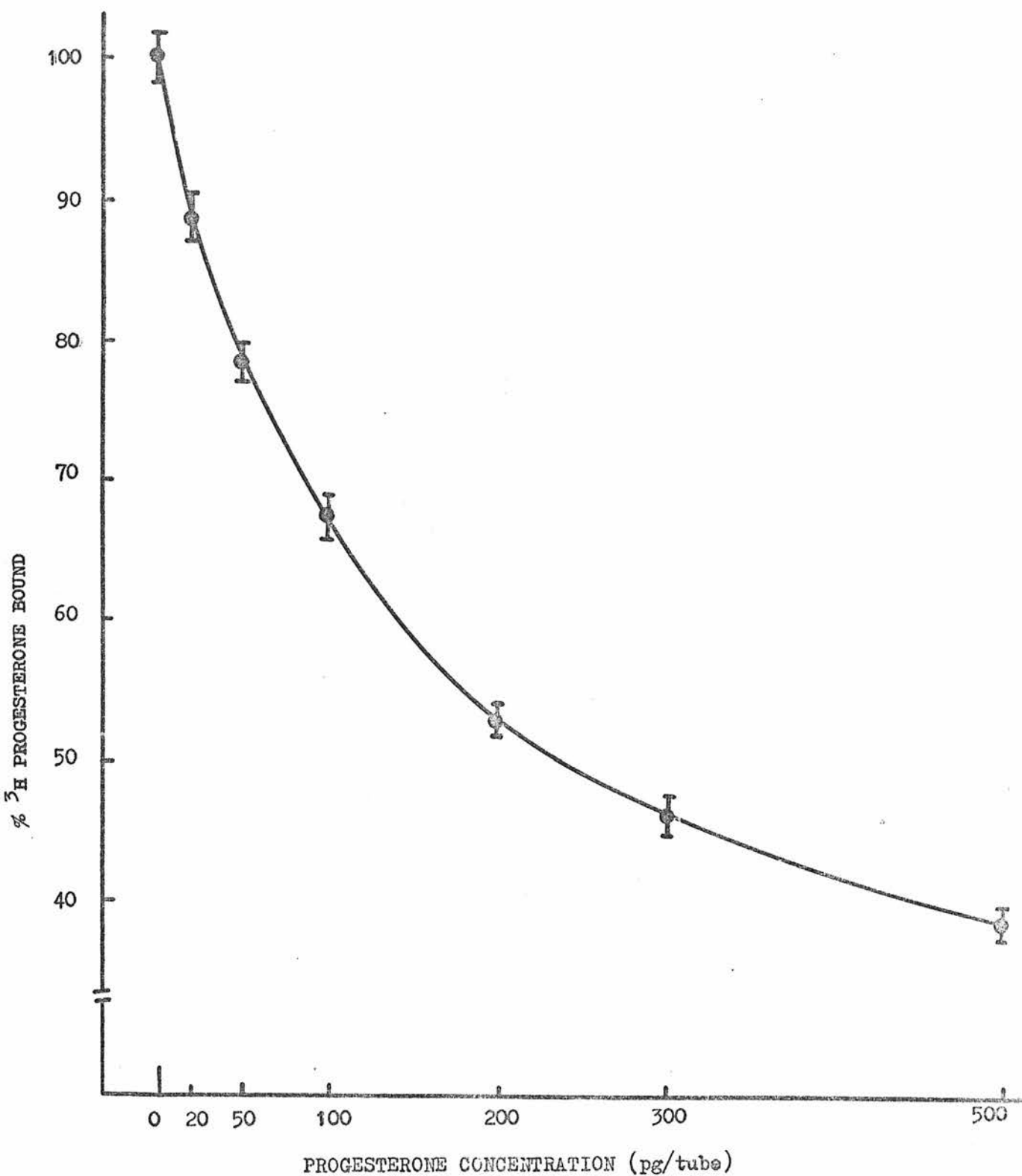
Specificity

The degree of cross reaction of 25 steroids in the system of the steroids tested was reported by Furr (1973), only the 11-hydroxypregn-4 ene-3, 20 dione epimers and 5α and 5β pregnone 3, 17-dione cross reacted significantly. Since less than 1% of the 11-hydroxypregn-epimers was extracted by light petroleum when added to plasma from castrated cockerels, it is unlikely that these steroids would have had a significant effect even if they were present in plasma.

Sensitivity and Precision

The means of 10 standard curves performed on 10 different assays are shown in Text - Figure 1. When no added unlabelled steroid was present the antibody bound 60 to 50% of the added radioactivity (3157 ± 40 (SE) cpm), which is expressed in Text - Figure 1 as 100% of the bound radioactivity. All points on this composite standard curve are significantly different from each other. 100 μl distilled water gave a concentration which never exceeded 5 pg/ml.

The variance within assays and between assays was evaluated from aliquots of 1 pool of plasma obtained from ewes on the day of parturition which were included in every assay. These samples gave a mean concentration of 0.38 ± 0.03 ng/ml and an interassay coefficient of variation of 16.3%.



Text-fig. 1 - Standard curve with antiserum (465/5) at dilution of 1:4000, the percentage of bound ^3H progesterone is plotted as a function of the amount of unlabelled progesterone in each tube. The curve shows the mean values \pm SE for 10 separate assays.

Prolactin Assay: A double antibody method similar to that of Arai and Lee (1967) and Buttle, Forsyth and Knaggs (1972) was carried out. Ovine prolactin NIH-P-B₂ was used as a standard preparation and as antigen.

First Antiserum - Antisera against prolactin were raised in guinea-pigs, and used for assay at a final dilution of 1:386000 which bound 48 ± 8 (S.D.)% of labelled prolactin.

Second Antiserum - A guinea-pig globulin fraction from non-immunised guinea-pigs was obtained by precipitating serum with 50% saturated ammonium sulphate followed by ion-exchange chromatography on DEAE cellulose (Whatman DE23) using 0.01 M-phosphate, pH 6.8, as eluting buffer and collecting the unretained protein fraction. The precipitating second antiserum was obtained by immunising rabbits with this guinea-pig globulin fraction. Under assay conditions guinea-pig globulin was included at 1.5 μ g/ml and the second antiserum added to a final dilution of 1:80.

Labelled Prolactin - Prolactin NIH-P-B₂ (10 μ g) was reacted with 1 mCi Na¹²⁵I (IMS 3 Radiobiochemicals, Amersham) by an enzymatic method (lactoperoxidase), a modification of the method of Morrison and Bayse (1970), and included in the assays at an approximate final concentration of 52 μ Ci/ μ g.

Standard prolactin solutions (corresponding to 0.9, 1.8, 3.5, 7.0, 15, 30, 60, 120 and 240 ng/ml) were set up in equal concentrations of plasma from hypophysectomised sheep for comparison with the plasma samples. As far as possible all samples from one sheep were included in one assay. In

each assay the prolactin content was determined in duplicate for each of two volumes of test plasma sample.

Tests for specificity, sensitivity and reproducibility of the assay showed that there is no cross reaction with 1 ug ovine, bovine or human growth hormones or human placental lactogen/ml (Buttle, et al., 1972). Analysis of variance of the \log_{10} transformation of prolactin concentrations of samples ranging from 30 to 600 ng/ml obtained from one goat showed that 98.2% of the total variation was attributable to the samples (Buttle et al., 1972).

Statistical analysis

The statistical techniques used were based on methods described by Snedecor (1956).

RESULTS AND DISCUSSION

CHAPTER 1

The Performance of the Ewes under Various Planes of Nutrition During Late Pregnancy

Introduction

This experiment was designed to determine the optimal energy requirements during late pregnancy and to measure the response to it in terms of ewe and lamb performance.

Attempts were also made to establish accurate measures of the optimal nutrients by analysing blood samples for blood metabolites irrespective of foetal load.

Experimental Design

Two experiments of six weeks duration each, were carried out during the months November-December 1971 and 1972. Pregnant Finn-Dorset ewes, which were 14 weeks pregnant, of approximately uniform body condition equivalent to grade 2.5 to 3.0 on the scoring system described by Russel et al. (1969), averaging 65 Kg live weight (range 49 to 83 Kg) and 3 years of age (2 to 5 years) were used. Three groups of 12 ewes each balanced for age, live weight and number of foetuses (determined by X-ray examination) were selected for the 1971 trial (Prefix 1) and two groups of 24 ewes each for the 1972 trial (Prefix 2). In each year the ewes within each group were allocated at random to the following dietary treatments:

1971 Treatment 1A - subjected to an ad-libitum feeding method.

1971 Treatment 1H - fed an energy level of 33 Kcal ME/Kg
live weight/day to meet the maternal

maintenance requirements plus a foetal allowance of 365 Kcal ME/Kg foetus/day (on the anticipated birth weight)

1971 Treatment 1M - was given 80% of the total energy which was fed to treatment 1H.

1972 Treatment 2H - was fed the same energy as treatment 1H in the previous year.

1972 Treatment 2L - was given 50% of the total energy which was allocated to treatments 1H and 2H.

The feeding scale and the daily nutrients intake for a 65 Kg ewe bearing twin lambs for each treatment are given in Table 1.1.

Table 1.1. - Details of feeding scale and the daily nutrients intakes employed during the last 6 weeks of pregnancy for a 65 Kg ewe bearing twin lambs.

Treatments	<u>Experiment 1 (1971)</u>			<u>Experiment 2 (1972)</u>	
	1A	1H	1M	2H	2L
No. of ewes	12	12	12	24	24
Feeds	250 g hay + concentrates			CRD	
Energy levels* (% of requirements)	ad-libitum	100	80	100	50
Daily nutrients intakes **					
Dry matter (g)	ad-libitum	1514	1211	2124	1061
Metabolizable Energy (Kcal)	"	4518	3614	4518	2259
Digestible Crude Protein (g)	"	168	134	242	121

* based on 33 Kcal/Kg ewe live weight + 365 Kcal ME/Kg foetus/day

** the daily intakes were based on the ewe body weight measured on two consecutive days at the time of allocation of individuals to treatment groups and on the foetal weight at birth assumed to be: single - 3.5 Kg; twin - 6.5 Kg and triplet - 8.2 Kg. The feed intakes were kept constant throughout the experimental period.

The feed levels were calculated per Kg ewe live weight and on anticipated foetal weight at birth (see Material and Methods). The diets were kept constant throughout the experimental period.

In both years, on 15th October, at 12 weeks of gestation, the ewes were brought indoors and penned individually and given hay ad-libitum until 14 weeks of gestation (1st November) when the animals balanced for age, live weight and number of fetuses were allocated to the different treatments and the experimental diets were introduced. For the 1971 experiment a feeding method was adopted in which the concentrate mixture was fed in addition to a basic 250 g/head/day of chopped hay. In the second experiment - 1972, the sole feed was a complete ruminant diet, given to all the ewes according to the experimental scale (Table 1.1).

The experimental procedure and chemical analyses of FFA, ketone bodies and glucose are listed in Material and Methods section.

RESULTS

Feed Intakes

The mean daily nutrient intakes for the different treatments during the last 6 weeks of pregnancy and mean weekly ME intakes are presented in Table 1.2 and illustrated in Fig. 1.1.

Although there was a considerable range of feeding levels (2.3 to 4.4 Mcal ME/ewe/day), no case of pregnancy

Table 1.2. The daily nutrient intakes, the blood metabolites concentrations and the ewes' performance during the last 6 weeks of gestation (Means and S.E.)

Treatment:	<u>Experiment 1 - (1971)</u>					<u>Experiment 2 - (1972)</u>			
	<u>1A</u>	<u>1H</u>	<u>1M</u>			<u>2H</u>	<u>2L</u>		
No. of Ewes:	12	12	12			24	24		
<u>Daily nutrient intakes</u>	<u>Mean</u>	<u>Mean</u>	<u>Mean</u>	<u>S.E.</u>	<u>Sig.</u>	<u>Mean</u>	<u>Mean</u>	<u>S.E.</u>	<u>Sig.</u>
Dry matter (Kg/ewe/day)	1.60	1.50	1.25	0.11		1.91	1.06	0.12	
Energy (Mcal ME/ewe/day)	4.39	4.12	3.49	0.12		4.09	2.27	0.07	
DCP (g/ewe/day)	170	156	135	5.4		192	111	3.4	
% of the allocated ration	ad lib	90.3	97.5	-		91	100		
<u>Plasma metabolites concentrations</u>									
Glucose (mg/100 ml)	70	71	64	1.8	**	55	46	1.3	***
FFA (μ equiv/litre)	231	372	532	36.9	***	582	995	34.9	***
Ketone bodies (ng/100 ml)	2.9	2.4	1.8	0.2	**	2.7	4.2	0.2	***
<u>Ewe body weight (Kg)</u>									
Mating	53	57	51	2.7	(N.S.)	66	65	1.7	(N.S.)
allocation to exp.	60	66	61	2.4	(N.S.)	68	69	1.6	(N.S.)
overall gain during gestation	14	15	16	1.3	(N.S.)	10	4	0.7	***
gain during last 6 weeks of gestation	7	7	6	1.2	(N.S.)	8	0	0.5	***
≠ Net body weight change	5	6	8	1.4	(N.S.)	0	-4	0.7	***
<u>Litter characteristics</u>									
No. of lambs/ewe	2.0	2.1	1.8			2.2	2.2	0.3	
Mean total lamb birth weight (Kg)	6.1	6.8	5.8	0.4	(N.S.)	7.4	7.1	0.3	(N.S.)
Mean lamb birth weight singles (Kg)	4.3	3.0	3.0	0.6		4.6	4.2	0.6	
Mean lamb birth weight twins (Kg)	3.2	3.5	3.3	0.4		3.8	3.4	0.6	
Mean lamb birth weight, triplets (Kg)	2.8	2.8	3.1	0.9		3.1	3.1	0.5	
Mortality 1st week of life (No. of lambs)	5	1	3			5	2		

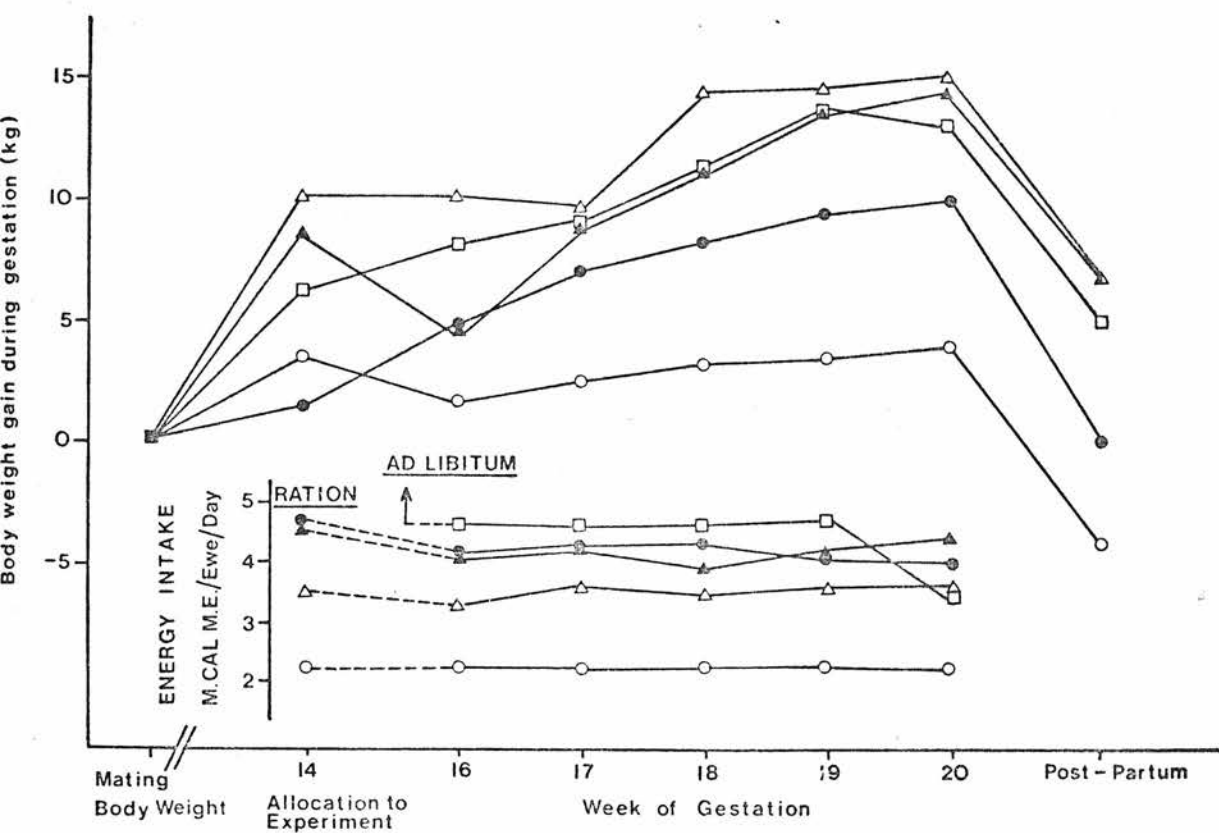
≠ difference between body weight at mating and body weight after lambing.

Analysis of variance, N.S. = non-significant, ** = $P < 0.01$ *** = $P < 0.001$

Text-figure 1.1.

Mean daily energy intake and the weekly live-weight changes during the last 6 weeks of gestation in 3 x 12 ewes which were fed a basal ration of 250 g hay/day supplemented by concentrates ad-libitum - treatment 1A (\square); to a 100% - 1H (\blacktriangle) and 80% - 1M (\triangle) of the maternal and foetal requirements.

2 other treatments of 24 ewes each were fed a complete ruminant diet at 100% level - 2H (\bullet) and 50% - 2L (\circ) of the maternal and foetal requirements.



toxaemia in late pregnancy occurred and no large quantity of feed was refused.

Decline in feed intake towards the end of pregnancy occurred in one group of ewes (treatment 1A) who were on ad-libitum feeding and showed a marked drop in the feed intake during the last few days of gestation. Before this drop, the mean daily voluntary intake of these ewes was very similar to that of the ewes in treatments 1H and 2H, but within the group the single bearing ewes consumed slightly more than ewes bearing twin or triplet lambs.

In the other treatments (1H, 1M, 2H and 2L), the ewes within each treatment were offered increasing amounts of feed according to the litter size. The intake data for single, twin and triplet bearing ewes show that within the range of this experiment most twin and triplet bearing ewes were able to consume all the feed offered. The small number of ewes which did not consume all their allocated ration were randomly distributed between the different treatments, size and number of foetuses.

Live-weight changes

The mean change in live-weight of the ewes are given in Table 1.2 and illustrated in Fig. 1.1. Body weight of the ewes from the three treatments in the 1971 experiment and those from treatment 2H in the 1972 experiment were not significantly affected by the feeding level and all gained between 6 to 8 Kg during the last 6 weeks of gestation while there was no change in the body weight of the ewes in treatment 2L which received 2.3 Mcal ME/ewe/day

during the same period. Also irrespective of the nutritional treatment, the loss of maternal live weight at parturition was similar for all treatment groups (8 to 10 Kg).

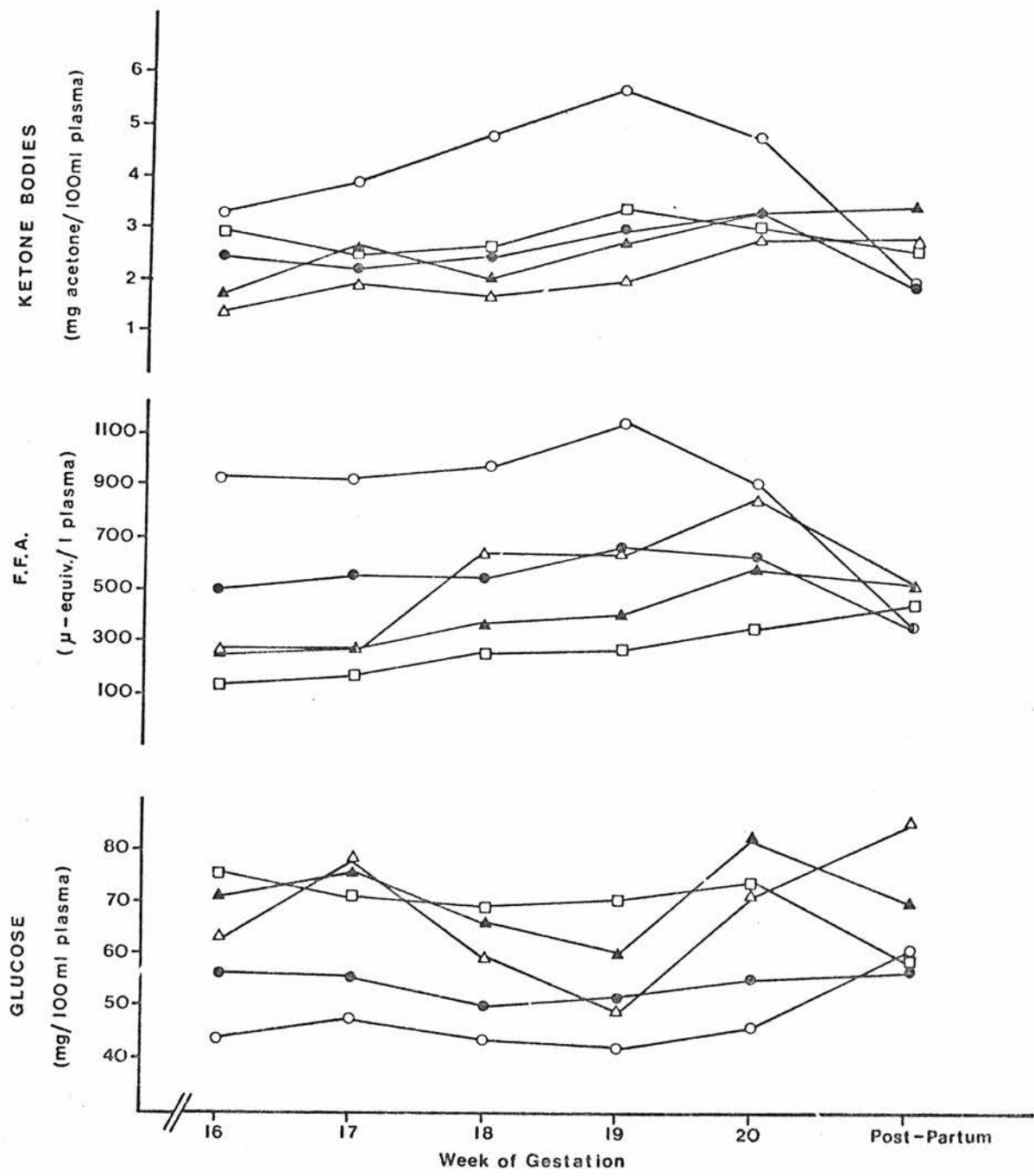
Abundant feeding during the last term of gestation as adopted in treatments 1A, 1H and 2H did not result in sufficient body weight gain to compensate for the weight loss at parturition. It is therefore of importance to note the overall change in weight of ewes throughout pregnancy which appears to be of more importance for a positive net body weight change from mating to parturition, as in the case of ewes of the 1971 experiment where there was a net gain of 5 to 8 Kg at parturition because of a body weight gain (7 to 10 Kg) during the first 14 weeks of gestation. The effect of the body weight gain in early pregnancy can also be seen in treatment 2L where although there was no body weight gain during the last term of gestation, the net body weight loss was only 4.0 Kg because of a body weight gain of 4.0 Kg in early gestation.

Blood Metabolites

The weekly mean concentration of peripheral blood plasma metabolites: FFA, ketone bodies and glucose (Figure 1.2), and their overall means for the last term of gestation (Table 1.2) reflect the nutritional and physiological changes in the ewe towards the end of gestation. The FFA concentrations in the peripheral blood plasma as an indicator of energy metabolism appears to be sensitive enough to demonstrate differences in energy intake between the treatments 1A, 1H and 1M which were not detected by the

Text-figure 1.2.

The weekly changes in peripheral plasma levels of glucose, non-esterified free fatty acids (FFA) and ketone bodies during the last 6 weeks of gestation in 3 groups of 12 ewes each which were fed a basal ration of 250 g hay/day supplemented by concentrates ad-libitum - treatment 1A (\square); to a 100% - 1H (\blacktriangle) and 80% - 1M (\triangle) of the maternal and foetal requirements. The 2 other treatments (24 ewes each) were fed a complete ruminant diet at a 100% - 2H (\bullet) and 50% - 2L (\circ) levels of the requirements.



changes in live-weight. In both years the FFA levels clearly differed between treatments although all increased slightly toward the end of pregnancy. Despite the constant feeding pattern employed, in the high energy treatments the FFA levels rarely exceeded 500 μ equiv/litre, a level considered to indicate undernutrition in the ewe (Russel, et al., 1967a). However, the much higher levels of FFA (995 ± 35 μ equiv/litre) in the blood plasma of the ewes in treatment 2L indicated undernourishment (Russel et al., 1967a).

The ketone bodies expressed as mg acetone/100 ml plasma (Table 1.2 and Fig. 1.2) increased towards the end of pregnancy and showed the same trend as that of FFA. In 1971 all treatments fell below 3 mg% and have no physiological significance. The degree of undernourishment imposed in treatment 2L was, however, sufficient to produce a consistent hyperketonaemia.

Blood glucose levels (Table 1.2 and Fig. 1.2) were significantly reduced in the lower dietary treatments (1M and 2L) but there were no significant differences between ewes bearing different litter sizes within treatments concerning these three metabolites except in 1A where a wide variation within the treatment was observed.

Litter Size

The performance of the ewes in term of total lamb birth weight and number of lambs born is given in Table 1.2. There were minor variations between the treatment groups in terms of the number of lambs born, total lamb birth weight, and the litter size. The differences in the mean lamb

birth weight were slightly larger in the second experiment but were not significantly so.

Intake of energy around recommended requirements on the one hand (treatments: 1A, 1H and 2H) and restricted feed intake (2L) on the other hand seems to have a very little effect on the lamb birth weight. Further, there was no suggestion that any of the dietary treatments influenced mortality during the first week of life.

Neither the nutrient intakes, mating body weight, body weight gain nor net body weight change were found to be related to number of lambs born or the total lamb birth weight.

Uniformity of nutritional status within treatments

Within treatments, the feeding of individual ewes according to the foetal load, as compared to ad libitum feeding, was very effective in keeping all the animals in one treatment on the same nutritional state irrespective of the no. of foetuses carried. This is well demonstrated in Table 1.3, where it can be seen that the plasma metabolite concentrations were similar for single, twin and triplets carrying ewes within the various treatments. This uniformity was achieved only partially in treatment 2L because of a few errors in pregnancy diagnosis which allowed excessive feed to be fed to the single bearing ewes.

Table 1.3. Means and S.E. of blood metabolites concentrations for ewes bearing single, twin and triplet lambs within treatments.

Treatment	No. of foetuses	No. of ewes	glucose (mg/100 ml)			FFA (μ equiv/litre)			Ketone bodies (mg/100 ml)		
			Mean	S.E.	Sig.	Mean	S.E.	Sig.	Mean	S.E.	Sig.
<u>Exp. 1 1971</u>											
1A ad libitum	Singles	3	71	3.6		260	74		2.8	0.5	
	Twins	7	75	2.4	***	169	48	*	2.5	0.3	*
	Triplets	2	52	4.4		403	90		4.2	0.6	
1H	Singles	1	80	6.2		554	128		1.4	0.8	
	Twins	9	70	2.1	(NS)	367	42	(NS)	2.3	0.3	(NS)
	Triplets	2	71	4.4		305	90		2.9	0.6	
1M	Singles	4	64	3.1		468	64		1.4	0.4	
	Twins	6	65	2.5	(NS)	536	52	(NS)	2.0	0.3	(NS)
	Triplets	2	62	4.4		646	90		2.2	0.6	
<u>Exp. 2 1972</u>											
2H	Singles	7	54	2.4		541	65		2.2	0.4	
	Twins	7	52	2.4	(NS)	631	65	(NS)	2.9	0.4	(NS)
	Triplets	10	58	2.0		577	54		2.9	0.3	
2L	Singles	5	48	2.8		794	76		2.9	0.4	
	Twins	10	43	2.0	(NS)	1054	54	*	4.8	0.3	**
	Triplets	9	47	2.1		1042	57		4.3	0.3	

DISCUSSION

In the present study, the different energy intakes did not affect the mean lamb birth weights, and the net body weight change of the ewe seems to be more affected by the overall gain during the whole gestation than by the body weight gain in the last term. The pattern of feeding during pregnancy has therefore little effect on either the overall live weight gain of the ewe during pregnancy or on the overall reproductive performance, as observed by Elsley, Bathurst, Cunningham, Dent, Dodsworth, Macpherson and Walker (1971) for the sow. The pattern by which feed is distributed during pregnancy may therefore be less important than the total feed given during gestation, although previous workers, e.g., Russel et al. (1967a); Davies, Johnston and Ross (1971) and Sykes and Field (1972) used or recommended (Robinson, et al., 1971) a low/high pattern of feeding during the late pregnancy which allows a progressive increase in intakes of energy and protein, a pattern which corresponds more closely to the demands of the developing foetus, which was shown by Wallace (1948) to increase three fold in weight between 16 to 20 weeks of pregnancy. In recent work, McClelland and Forbes (1973) found that ewes on low/high energy intakes had a significantly lower net body weight loss than did ewes on constant energy intakes, but again the pattern of feeding had no significant effect on lamb birth weight.

The mean daily energy intake of 4.2 Mcal ME/ewe fed to treatments: 1A, 1H and 2H for the last six weeks of gestation is above the energy level recommended by Russel

et al. (1967b), but similar to the average of 4.07 Mcal ME/ewe/day calculated from the data of Robinson et al. (1971) who recommended an energy level of 3.6 Mcal ME/ewe/day at 55 days prepartum rising progressively to 5.3 Mcal ME/ewe/day five days pre-partum for an 80 Kg ewe producing twin lambs of 8.4 Kg. Nevertheless, this high energy intake had no beneficial effect on the lamb birth weight and a positive net body weight change at parturition was obtained only if there was a considerable gain during early pregnancy. These results are in good agreement with the results obtained with Scottish Blackface ewes in which a higher level of nutrition during the final 7 weeks of pregnancy failed to increase single lamb birth weight and to overcome the effect of loss of weight at earlier stages of gestation (Russel and Foot, 1972).

The minimal effect of nutritional level on lamb birth weight, of the different groups, is in accordance with previous findings of Robinson and Forbes (1968); Robinson et al. (1971); Davies et al. (1971) and McClelland and Forbes (1971, 1973). Russel et al. (1967a,b) however, reported that undernourishment during the latter half of pregnancy reduced birth weight of single and twin lambs and that a very high correlation exists between mean daily D.O.M. intake during 15 to 6 days pre-partum and foetal weight at term. A highly significant relationship between FFA and between ketone bodies and lamb birth weight was also reported by Sykes and Field (1972). A comparison of the present data with previous studies on the effect of metabolizable energy ($\text{Kcal/Kg}^{0.73}/\text{day}$) and digestible crude protein ($\text{g/Kg}^{0.73}/\text{day}$)

Table 1.4. Effect of intake of metabolizable energy (Kcal/Kg^{0.73}* /day) and digestible crude protein (g/Kg^{0.73}/day) during gestation on ewe and lamb performance.

Treat.	Robinson & Forbes 1968		Sykes & Field 1972		Present Data				
	E ₁ P ₁	E ₂ P ₂							
					ad-libitum	LH	LM	2H	2L
ME	142	124	-		221	194	174	188	103
DCP	4.6	5.1	82		8.6	7.3	6.7	8.8	5.0
Ewe Wt. change (%)	+1.4	-0.7	-18.0		+6.8	+7.5	+11.3	0	-5.0
Lamb birth weight** (g/Kg ^{0.73})	21.2	20.2	20.8		15.6	15.0	16.0	15.4	14.6

* Ewe body weight at allocation to experiment.

** Robinson and Forbes' data was corrected to the basis of twin bearing ewes.

Table 1.5. Effect of intake of metabolizable energy (Kcal/Kg/day)* on
Total lamb birth weight (g/Kg)

Russel et al. 1967**		Present data				
Group	II	III	ad-libitum	LH	LM	2H 2L
Singles						
Number	13	8	3	1	4	7 5
ME	52	34	81	68	52	58 26
TLBW	92	76	75	54	47	71 58
Twins						
Number of pairs	6	8	7	9	6	7 10
ME	66	48	73	64	59	62 33
TLBW	142	116	105	105	112	117 106
Triplets						
Number of set	-	1	2	2	2	10 9
ME	-	52	61	55	59	62 38
TLBW	-	150	131	122	141	130 128

* Based on the body weight at allocation to the experiment.

** The intake in Russel's data refers to mean daily intake on days 6 to 15 pre-partum.
The present data refers to mean daily intake during the last 6 weeks of gestation.

intakes during gestation on ewe and lamb performance is presented in Tables 1.4 and 1.5. The conflicting results confirm the limitation of use of foetal weight as an index of the adequacy of level of nutrition in late pregnancy as previously discussed by Russel et al. (1967a).

The method of feeding ewes within treatments according to the number of foetuses (determined by X-ray at 90 days of gestation) used in the present study avoided the variation in nutritional state which arises from differences in foetal number (Russel et al. 1967a), and led to a relatively uniform performance within the treatment groups. The method seems to be more easily practiced than the feeding to pre-determined levels of FFA and ketone bodies developed by Russel et al. (1967a) and used by Sykes and Field (1972).

The inverse relationship between FFA levels and the energy intake, confirms the use of FFA as an index of the nutritional state even in cases where high energy levels are fed. The higher FFA values found in ewes of treatment 2H than in treatment 1H, when the energy levels were similar for both treatments, could have arisen either from the low glucogenic content in the complete ruminant diet used in treatment 2H (Annison, 1960; Reid and Hinks, 1962a), or from the inferior body condition of the ewes in treatment 2H during the pre-experimental period.

CHAPTER 2

The use of Dexamethasone for Inducement of Parturition

Introduction

From the literature cited (see Literature Review, Section A3) it was concluded that in the later stage of pregnancy, parturition can be successfully induced by administration of glucocorticoids to farm animals. The present experiment was conducted to observe the effects of a single injection of dexamethasone (16 mg) given intramuscularly to Finn x Dorset ewes which were maintained on 2 planes of nutrition during late pregnancy and carried 1-4 foetuses.

Experimental

Forty-five ewes taken from treatments 2H and 2L (for details see Chapter 1) were used for synchronisation of lambing by intramuscular injection of dexamethasone on 141st day of gestation between 20.00 and 21.00 hours. The ewes in two groups of 21 (treatment 2H) and 24 (2L) were fed CRD at 2 levels - an average of 4.5 Mcal/ewe/day and an average of 2.3 Mcal/ewe/day respectively. A group of 40 ewes from the same flock that were in conventional farm management were not injected. They were fed hay (ad-libitum) and concentrate (750 g/ewe/day) during the same period (last 6 weeks of gestation) and served as a control group. After treatment the ewes were carefully observed, births were attended and assisted where necessary, and parturition time and lambing data recorded. After lambing, ewes were

checked for the initiation of milk secretion and weight gain of lambs from the treated and non-treated ewes were analysed.

RESULTS

The mean time of parturition was 49.0 ± 2.18 hours after the injection of dexamethasone (Table 2.1). This resulted in a mean gestation length of 143.3 days in the treated group, compared to 144.8 in the control ($P < 0.05$). Of greater importance is the spread of lambing in the treated group, as can be seen from Figure 2.1. The variance is 0.355 days^2 compared to 4.346 days^2 in the control group - a 12.2 fold difference. 98% of the treated ewes lambed by the 144th day of gestation compared with 95% on the 148th day in the control group (Figure 2.1).

Nutritional treatment had no effect on the synchronisation of lambing and the duration of gestation and was therefore ignored.

The spread of parturition within the 24 hours of the day was equally distributed between day (06.00 to 18.00) and night (18.00 to 06.00) (Table 2.1). The gestation length was slightly shorter in ewes carrying triplets or quadruplets. Treated ewes carrying triplets or quadruplets lambed on an average of 10 hours earlier than those carrying single or twin foetuses. The same trend could be noted in the control group. Age was found to have little effect on the response to the dexamethasone treatment (Table 2.1).

No ill effects in the ewes or lambs born were observed in the treatment groups; no placental retention was observed, milk secretion was initiated in all the injected ewes immediately after lambing, and no case of stillbirth

Table 2.1. Intervals between injection and parturition in Finn x Dorset ewes treated with a single dose of dexamethasone (16 mg. intramuscularly) on 141st day of gestation

Group	No. of lambs per ewe	No. of sheep	Time interval injection-parturition (hr)		Length of gestation (days)			Time of parturition day (06.00-18.00hr) (18.00-06.00hr)	
			Mean	S.E.	Sig.	Mean	S.E.	Sig.	(%)
Treated	1-2	29	52.9	2.40	**	143.5	0.15	-	-
	3-4	16	41.9	3.80		143.0	0.13	-	-
	<u>1-4</u>	<u>45</u>	<u>49.0</u>	<u>2.18</u>		<u>143.3</u>	<u>0.09</u>	<u>51</u>	<u>49</u>
Control	1-2	29	-	-		144.9	0.36	-	-
	3-4	11	-	-		144.3	0.78	-	-
	<u>1-4</u>	<u>40</u>	-	-		<u>144.8</u>	<u>0.33</u>	<u>60</u>	<u>40</u>

Student t test: * = $P < 0.05$, ** = $P < 0.01$

+ difference between treatment means significant at $P < 0.05$
(Fisher-Behrens test)

Differences between 'number of lambs' groups tested for significance
by student t-test: * = $P < 0.05$, ** = $P < 0.01$.

Text-figure 2.1.

Induction of Parturition by an injection
(16 mg) of Dexamethasone on the 141st day
of gestation in Finn x Dorset ewes.

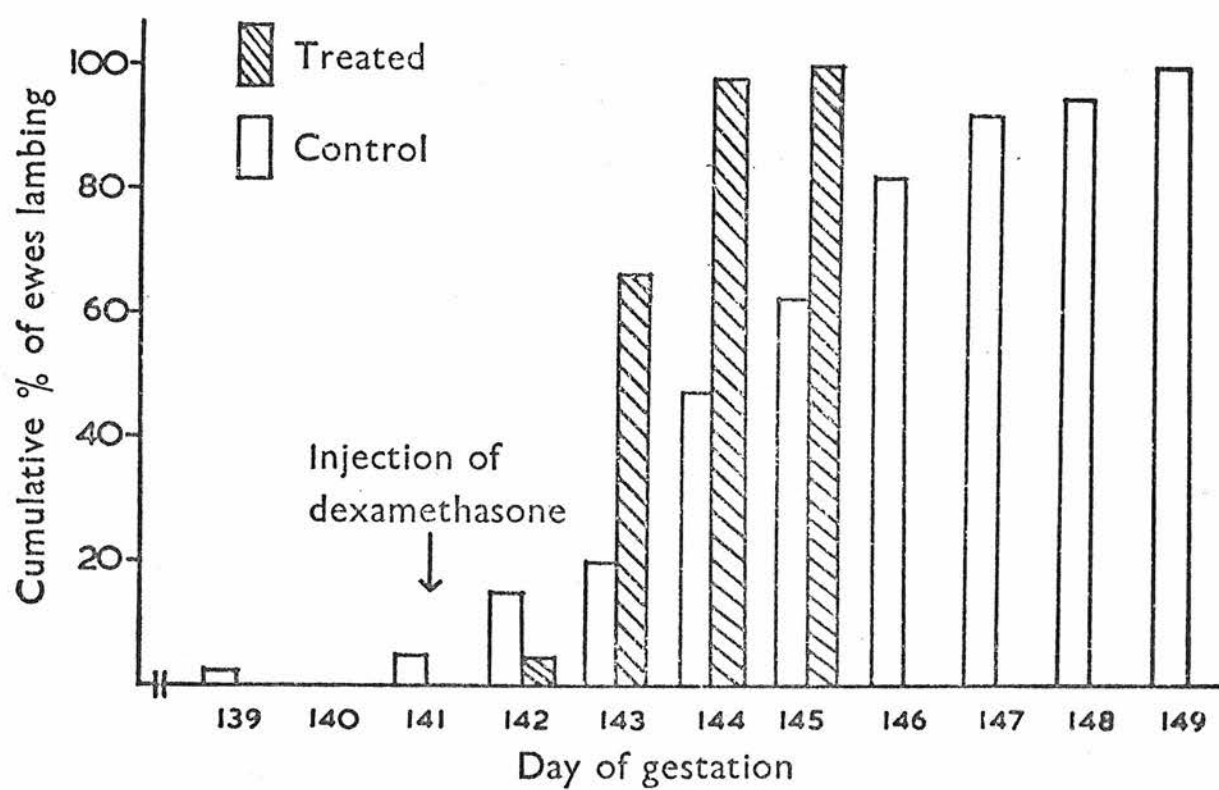


Table 2.2. Increase in weight of newborn lambs after induction of parturition by dexamethasone

Group	No. of ewes	No. of lambs per ewe		Lamb birth weight (kg)		Weight at 8 weeks of age (kg)		Mortality 1st week (%)
		Mean	SE†	Mean	SE†	Mean	SE†	
Treated	45	2.13	0.08	3.59	0.13	15.8	0.57	5
Control	40	2.07	0.08	3.43	0.08	14.7	0.57	9.5

+ Treatment differences were not significant.

was recorded after treatment. Lamb viability and performance (Table 2.2) was good; mortality rate during the first week of life was 5% in the treated group compared with 9.5% in the control.

DISCUSSION

The spread of lambing around the average parturition date varies within breeds as well as between breeds (Bradford, Hart, Quirke and Land, 1972). Although the techniques of synchronisation of oestrus at mating permit a concentration of parturition, the period of time in which lambing takes place varies greatly. A reduction in this period, therefore, could lead to a decrease in observation outside working hours and possibly a reduced mortality during the lambing period. In this experiment a single injection of dexamethasone at a chosen dose level (16 mg intramuscularly) near the normal full term of gestation (4 days before the mean gestation length) appeared to be safe to initiate labour and the technique is simple. The feed intake level during late pregnancy and the foetal load had no effect on the ewes' response to the induced treatment. This indicates that dexamethasone can be used to induce parturition successfully even in ewes subjected to poorer nutritional conditions during late pregnancy and carrying between 1 and 4 foetuses. Also the time of day in relation to the injection did not seem to have any effect on the spread of lambing which was equally distributed between day and night in both treated and untreated ewes. This

contrasts with the results of Bosc (1972) who reported a tendency for parturition to occur in day or night according to the time of injection in Île de France and Préalpes ewes. Unlike the cow, where placenta retention was found to be a very common occurrence when parturition was induced with dexamethasone (Jochle, Espanza, Gimenez and Hidalgo, 1972), this was not the case in this experiment. However, a higher proportion of assisted births either by correcting the lamb's position or by applying traction to assist or to complete delivery of the foetus (distocia) occurred in the dexamethasone treated ewes, but this may have been the result of undue concern for the treated ewes.

CHAPTER 3

The Plasma Concentration of Progesterone, LH and Prolactin during Late Pregnancy and Induced Parturition

Introduction

Previous studies reported that the plasma progesterone concentrations were related to the number of fetuses carried by the ewe and that the concentrations were elevated in undernourished animals (see Literature Review, Section B2.2). The present experiment was designed to study the combined effects of nutrition and litter size on the plasma circulating levels of progesterone, LH and prolactin during late pregnancy and at induced parturition by an injection of dexamethasone.

Experimental

The hormones were measured in blood plasma samples taken from 48 ewes that were on treatments 2H and 2L (described in Chapter 1) on Days 100, 120, 134, 141 of gestation and on Day 143 (the day of parturition). As described earlier, the ewes within each group were individually penned and fed a complete ruminant diet (CRD) incorporating 40% straw, and containing 1.8 Mcal metabolisable energy (ME) and 9.0% digestible crude protein (DCP) at two levels. The first of these, treatment 2H, provided a daily energy level of 33 Kcal ME/Kg ewe live weight, designed to meet the maternal maintenance requirements, plus a foetal allowance of 365 Kcal ME/Kg foetus/day (on the anticipated foetal weight at birth). The second, treatment 2L, provided half the total energy allocated to treatment 2H.

In this way it was intended that the nutritional state of the ewe was not influenced by the number of fetuses carried. These two energy levels were kept constant throughout the experiment.

Further details of the 2 treatments are given in Chapter 1; details of the induced parturition treatment in Chapter 2 and details of the hormone assay techniques in Material and Methods.

RESULTS

The nutritional state

Successful adjustment of the diet of the ewes to eliminate the effects of litter size within the high and low treatments was of critical importance, and was therefore verified by the measurement of plasma FFA concentration as an indication of nutritional state (Patterson, 1963), and the measurement of live weight changes. These are given in Table 3.1. The mean plasma FFA concentration was similar for ewes carrying 1, 2 or 3 fetuses within the high treatment, and although litter size did have an effect within the low treatment, there was a much greater difference between treatments ($P < 0.001$). Similarly, the ewes on the high energy diet gained an average of approximately 8 Kg during the last 6 weeks of gestation, whereas those on the low diet only maintained their body weight. These observations therefore indicate that the treatments had maintained a relatively uniform nutritional state within treatments, but had induced differences between treatments.

Table 3.1. Mean feed intake and the performance (\pm S.E.) of pregnant ewes on two nutritional levels which were adjusted according to litter size during late pregnancy

Treat- ment	Foetal load	No. of ewes	Energy intake in Mcal ME/day	Body weight gain ^a in Kg	Plasma FFA (μ equiv/ litre) Day 100	Plasma FFA ^b (μ equiv/ litre)		Total lamb birth weight in Kg
						Day 100 -	Parturition	
2H	Single	7	3.8 \pm 0.1	7 \pm 0.9	766 \pm 14	541 \pm 65		4.6 \pm 0.6
	Twin	7	4.0 \pm 0.1	9 \pm 0.9	874 \pm 10 (N.S.)	631 \pm 65 (N.S.)		7.6 \pm 0.6
	Triplet	10	4.4 \pm 0.1	9 \pm 0.8	872 \pm 10	577 \pm 54		9.2 \pm 0.5
	Total	24	4.1 \pm 0.07	8 \pm 0.5	851 \pm 6	582 \pm 35		7.4 \pm 0.3
2L	Single	5	1.9 \pm 0.2	-3 \pm 1.1	721 \pm 13	794 \pm 76		4.2 \pm 0.7
	Twin	10	2.1 \pm 0.1	0 \pm 0.8	913 \pm 12 (N.S.)	1054 \pm 54 *		6.8 \pm 0.5
	Triplet	9	2.7 \pm 0.1	2 \pm 0.8	834 \pm 10	1042 \pm 57		9.2 \pm 0.5
	Total	24	2.3 \pm 0.07	0 \pm 0.5	829 \pm 11	995 \pm 35		7.1 \pm 0.3

^a total gain during the last 6 weeks of gestation

^b calculated from the mean of six weekly means

* P < 0.05 (Analysis of variance)

The plasma hormone levels

Mean progesterone, LH and prolactin concentrations at different times of gestation, in the plasma of 48 ewes each carrying 1 to 3 foetuses and maintained on two nutritional treatments are given in Table 3.2 and illustrated in text-fig. 3.1.

Progesterone

The presence of twin and triplet lambs markedly influenced the progesterone concentrations in the plasma samples obtained from 48 pregnant ewes grazing on natural pasture on the 100th day of gestation. The mean plasma progesterone for the 36 ewes carrying twins or triplets were significantly greater ($P < 0.001$) than in the 12 ewes carrying a single foetus (Table 3.2.).

The mean plasma progesterone concentrations of all animals in the high nutrition group (2H) was lower than that of those in the low intake group (2L) between Day 100 and parturition. The mean plasma progesterone concentration on the 120th day of gestation was 17.8 ± 0.95 ng/ml in the restricted ewes compared with 14.2 ± 0.95 ng/ml in the well fed ewes ($P < 0.001$). Within each treatment, however, the differences between single, twin and triplet bearing ewes were much less than on Day 100, especially in the high group, and non-significant in both cases. In the two treatments the progesterone values measured within hours after induced parturition were in most ewes below 1 ng/ml.

The relationships between litter size, plasma progesterone and plasma FFA concentrations were studied by

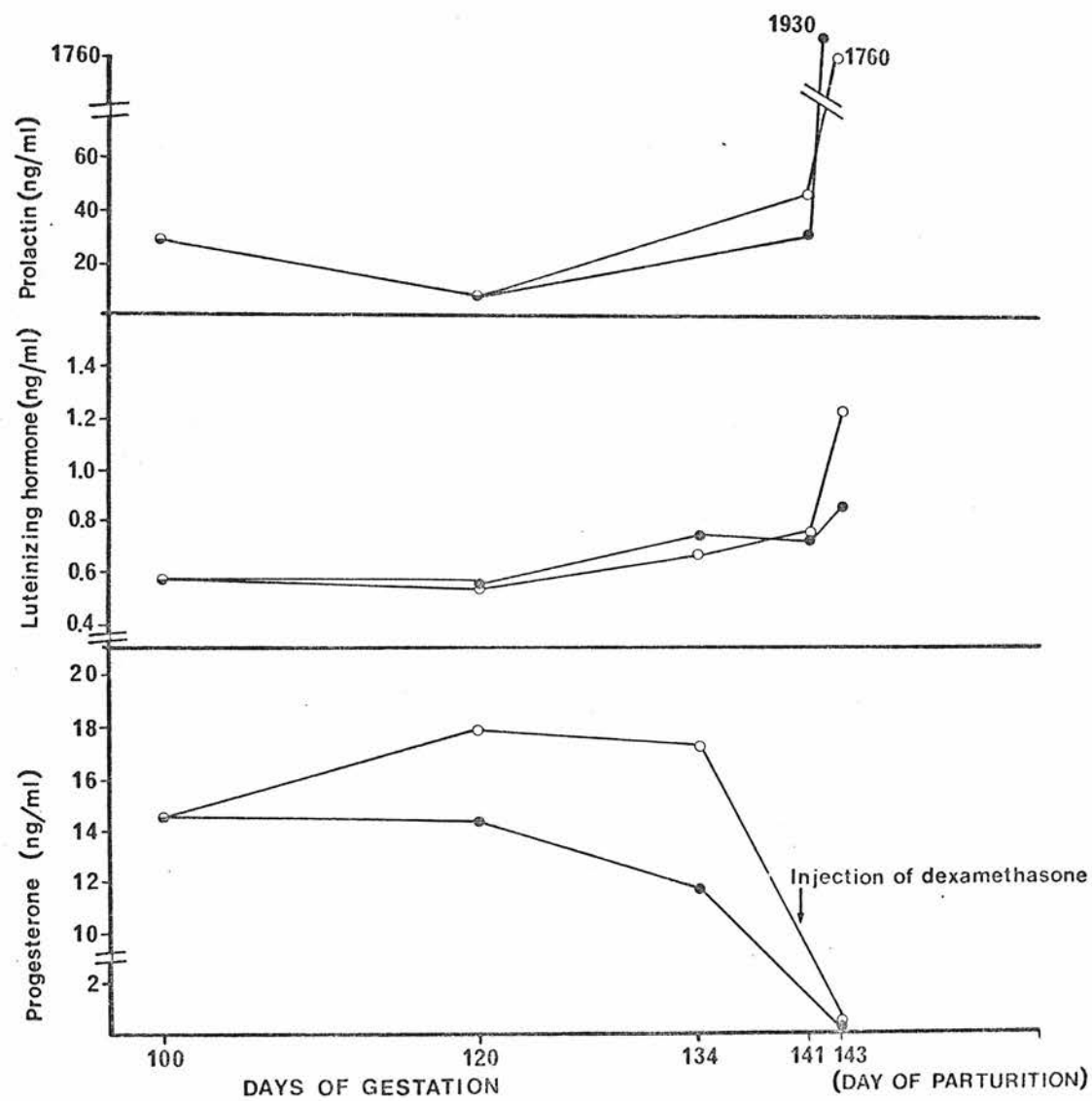
Table 3.2. Progesterone, LH and prolactin concentrations in the peripheral plasma of pregnant ewes measured before and after allocation to two planes of nutrition and after induced parturition

Day 100 to parturition												
Hormone measured on day of gestation												
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Analysis of variance, \dagger = N.S.; *** = P < 0.001.

Text-fig. 3.1.

Plasma progesterone, LH and prolactin measured at different times during late pregnancy in ewes 2H (●) and 2L (○) before and after allocation to two nutritional treatments at 100 days of gestation and after dexamethasone induced parturition.



least square analysis. At Day 100, litter size had a highly significant effect on progesterone concentration ($F = 7.883$ on 3 and 43 d.f.; $P < 0.001$) but had no significant effect on FFA levels. Conversely the regression of litter size on progesterone level was also highly significant ($F = 25.48$ on 1 and 43 d.f.; $P < 0.001$), suggesting that it would be a useful predictor of litter size. The regression of litter size on FFA was not significant. On Days 120 and 134 of gestation, however, when a uniform state of nutrition was achieved within treatments, there was no evidence that litter size affected either progesterone or FFA levels. Nor was progesterone a good predictor of litter size any longer, either within diets or pooled over diets.

A highly significant correlation was found between progesterone and FFA concentrations before and after allocation to the nutritional treatments. The pooling of sums of squares and cross products calculated within litter sizes, gave correlations above 0.9 for Days 100, 120 and 134 in both treatments, showing that the relationship between plasma progesterone and FFA is not dependent on litter size.

Luteinizing hormone

LH concentration showed a different pattern from that of progesterone. The concentrations in the maternal plasma did not differ significantly between the high and low treatments, nor did the number of foetuses carried by the ewe influence the maternal LH level. The concentrations remained in the range of 0.4 to 1.0 ng/ml throughout the last

term of gestation, with a slight but non-significant rise, towards the end of gestation and after dexamethasone induced parturition.

Prolactin

Considerable variation was found in the plasma prolactin concentration in samples taken from 18 of the ewes on days: 100, 120, 141 of gestation and on the day of parturition. The mean plasma prolactin concentrations for the two treatments were found to decline slightly on Day 120 and then increased very rapidly from Day 141 to Day 143, the day of parturition.

The considerable variation among animals concealed any possible effects of nutrition or foetal load on the maternal plasma prolactin level.

DISCUSSION

The plasma progesterone concentrations measured in the present study using a radioimmunoassay technique were similar to the values obtained by previous workers who used competitive protein binding techniques: Bassett et al. (1969); Fylling (1970); Obst, Seamark and McGowan, (1971) and Stabenfeldt, Drost and Franti (1972). These studies established a gradual increase in the plasma progesterone concentration in pregnant sheep up to 130 to 140th day of pregnancy and a declining before the end of gestation to almost zero immediately after parturition. However, considerable variation, among animals in the magnitude of the increase and the time of decline before parturition was detected.

In the present study the progesterone levels measured on the 100th day of gestation, before allocation to nutritional treatments, were significantly affected by the number of lambs, as observed by Bassett et al. (1969) and Gadsby, Heap, Powell and Walters (1972), who were able to predict the number of fetuses with 69% accuracy for litters of 0, 1 to 2 and 3 to 4 in an intensively housed flock of sheep, 91 to 105 days after mating. However, at this stage plasma progesterone concentrations were also significantly correlated with the plasma FFA which indicates that the variation in progesterone does not simply arise from variation in litter size alone and other factors such as nutrition may be involved. An increase in plasma progesterone during undernutrition of the ewe in early pregnancy was reported by Cumming et al. (1971). When the effects of nutrition were removed biologically by adjusting the diet to the demands of the fetus, the regression between progesterone concentration and litter size disappears, but a strong correlation between progesterone and the concentration of FFA remained. It is therefore only possible to conclude that the concentration of plasma progesterone is associated both with litter size and with FFA concentration, and that these associations may interact with both the stage of gestation or the level of nutrition. Thus the higher progesterone levels found in the low fed group (2L), combined with the absence of a relationship between progesterone and litter size when the ewes in both groups achieved a uniform body condition, stress the effect of nutrition on the maternal plasma progesterone and indicate the possible use

of plasma progesterone as a possible criterion for the assessment of nutritional state in sheep.

Cumming et al. (1971), suggested that the increase in plasma progesterone may be due to decreased metabolic rate, mobilization of stores of progesterone or increased rate of secretion. However as the change in plasma progesterone was not accompanied by a change in LH or ACTH (Cumming et al., 1971), or LH or prolactin (present data), the cause of the rise in plasma progesterone during under-nutrition in the ewe is not evident.

From the recent studies on the importance of the secretion of cortisol by the foetal adrenal for the initiation of labour (Liggins, 1968; Liggins, Grieves, Kendal and Knox, 1972), it is clear that progesterone should be viewed as only one of the factors associated with the process of parturition. In the present study, parturition was stimulated artificially by an injection of dexamethasone on the 141st day of gestation, parturition took place within 49 ± 2.18 hours after injection (reported in Chapter 2) and was preceded by a decline in maternal progesterone to well below 1 ng/ml on the day of parturition (Table 3.2). This was consistent with previous findings in sheep (Fylling, 1971) and cattle (Schams, Hoffman, Fisher, Marz and Karg, 1972), who reported that 10 mg flumethasone induced parturition which was preceded by a marked decrease of progesterone similar to that observed before a normal delivery. Nutritional treatments similar to those adopted in this experiment did not have any effect on the

events associated with the initiation of induced parturition.

LH may play a major role in maintenance of luteal function in the ewe as discussed by Niswender et al. (1968), then it might be expected LH will be detectable in the plasma of the pregnant ewe, but the present data and others in sheep (Niswender et al., 1968; Davis et al., 1971) and cattle (Oxender, Hafs and Edgerton, 1972; Schams et al., 1972) showed that LH levels remained below 1 ng/ml throughout pregnancy and parturition and are not affected by foetal number. A very sharp and brief pulsatile release of LH in the blood of a pregnant Zebu cow with rapid return to baseline values was however reported by Carr (1971). Chamley, Buckmaster, Cerini, Cumming, Goding, Obst, Williams and Winfield (1973), reported higher levels of LH (2.0 to 6.0 ng/ml) in 5 ewes bled from -36 to +36 hours after parturition but no LH surge was released at the time. Chamley et al. (1973), postulated that the failure of progesterone to fall and the rise in oestradiol 17 β to evoke an LH surge at parturition is due to a low pituitary content of LH at a time of parturition.

LH showed a different pattern of secretion from that of progesterone throughout the period of study, and no direct relationship can be seen in the two hormones. Thus it is still to be explained why the basal levels of LH in peripheral blood remains constant around parturition when major changes in other hormonal relationships are known to occur.

The changes in prolactin levels in blood plasma before and after parturition observed in the present study, agrees with previous observations in sheep (David et al., 1971;

Chamley et al., 1973), cow (Schams and Karg, 1970; Oxender et al., 1972) and goat (Buttle et al., 1972; Hart, 1972). In these species serum prolactin stabilized at ^a low level during early and mid pregnancy, ^{and} appeared to gradually increase 3 to 5 weeks prior to parturition, then ^{to} increased rapidly to its highest level on the day of parturition.

Schams and Karg (1970) suggested that the rise in plasma prolactin before parturition is associated with parturition and not with the onset of lactation. Thus it is possible that the very high prolactin concentration measured after parturition in the present study may reflect the stress of lambing or result from non-specific stress as observed in cows (Tucker, 1971).

CHAPTER 4

The Effect of Lactation on the Resumption of Reproductive Activity in Post-Partum Ewes

Introduction

The resumption of cyclic reproductive activity during different periods of lactation was studied, with particular emphasis on the pre-ovulatory release of LH at synchronised oestrus 35 days post-partum, in ewes, where the effects of seasonal and dietary deficiency factors had been eliminated as far as possible, as discussed in the literature review Section A4.

Experimental

Fifty Finn-Dorset (F_1) ewes were divided after lambing at random into 3 groups, two of 17 and one of 16. One group of 17 ewes had their lambs weaned at 1 day of age, whereas the other two groups of 16 and 17 ewes suckled twin lambs for 24 and 56 days respectively. All ewes lambed during the 3rd week of December 1971, well within their normal breeding season and all were in good body condition.

The ewes were housed in 3 separate pens and fed hay (5% DCP and 1.8 Mcal ME/Kg) and concentrate (10% DCP and 2.6 Mcal ME/Kg) ad-libitum. The lambs had access to creep feed. The nutritional status of the ewe was monitored by weekly analysis of blood FFA and body weight changes.

Spontaneous oestrus occurring during the first 3 weeks post-partum was checked with vasectomized rams twice daily at 09.00 and 16.00. At the end of the third week a progestagen treatment was effected using intravaginal sponges

inserted for 12 days (see Material and Methods).

Twenty-two hours after sponge withdrawal, rams were introduced to the ewes for 15 minutes and then again at 2 hourly intervals for further 15 minutes until oestrus was detected, up to a limit of 50 hours after sponge withdrawal.

The time of onset of oestrus and the discharge of LH were studied in 12 ewes of the 1 and 56 day lactation groups and 11 of the ewes which lactated for 24 days. After oestrus was detected the ewes were removed to individual pens and blood samples were then taken from each ewe at 2 hourly intervals from 2 to 30 hours after the onset of oestrus. The blood samples were treated before LH analysis as described in Material and Methods.

The number of CL on each ovary was counted by laparoscopy in a sample of 15 ewes two weeks after parturition, and in all the 35 ewes on the 8th day of the synchronised cycle.

RESULTS

Energy intake and ewe performance

The nutritional status of the ewes was assessed by measuring the FFA in the peripheral plasma and the body weight gain during the period of the experiment. The results represented in Table 4.1 indicate that the body weight gain was similar for ewes which lactated for either 1 day or 56 days. (The smaller increase in body weight of the 24 day lactating group could have arisen from feed restriction at the time of weaning). All the ewes lactating for 56 days either maintained or gained weight, three of the

Table 4.1. Voluntary energy and protein feed intakes, body weight gain and non-esterified free fatty acids in the peripheral blood during the first 5 weeks after lambing of ewes completing 24 or 56 days of lactation

Duration of lactation (days)	No. of ewes	Daily feed Energy (Mcal ME/ewe)	Protein intake (g DCP/ewe)	Mean body wt after lambing in kg \pm S.E.	Mean body wt gain in kg/ewe \pm S.E. (range in parentheses)	Mean plasma FFA in μ equiv/litre \pm S.E. (range in parentheses)
1	17	5.2	200	60.2 \pm 1.5	6.4 \pm 1.3 (-4to+14)	181 \pm 13 (113 to 316)
24	16	6.4	240	62.0 \pm 2.3	3.2 \pm 0.8 (-3to +6)	403 \pm 27 (275 to 646)
56	17	6.9	260	62.7 \pm 2.6	5.2 \pm 0.9 (0to+13)	446 \pm 46 (156 to 676)

ME, metabolic energy; DCP, digestible crude protein;
FFA, free fatty acids

early weaned (1 day) group lost weight as did three of the 24 day group. In terms of body weight changes therefore there was no indication of nutritional stress in the lactating ewes relative to the dry ewes. This was confirmed by the observation that the mean FFA concentrations in the peripheral plasma of all three groups were below the range of 500 to 1200 μ equiv/litre considered to indicate undernourishment in the ewe (Patterson, 1963; Russel, Doney and Reid, 1967a).

Post-partum interval to first oestrus

Eight of the seventeen ewes whose lambs were weaned at 1 day of age showed oestrus during the first 3 weeks after lambing compared to only one of the thirty-three lactating ewes, showing that the incidence of oestrus is greater in the dry ewes. The results of laparoscopy during the 3rd week after lambing indicated that 'silent' ovulations did not occur during this period, and all the ewes which had shown oestrus did ovulate. The summary of body weight gain during this period (Table 4.2) indicates that there was no difference between the dry and lactating ewes. Furthermore, the sub-division of the ewes into those which showed oestrus and those which did not, indicates that the gain was smaller in those ewes showing oestrus. The individual weight changes and FFA concentrations are presented in text-figure 4.1, where it can be seen that the display of oestrus was not associated either with body weight change or with FFA concentration.

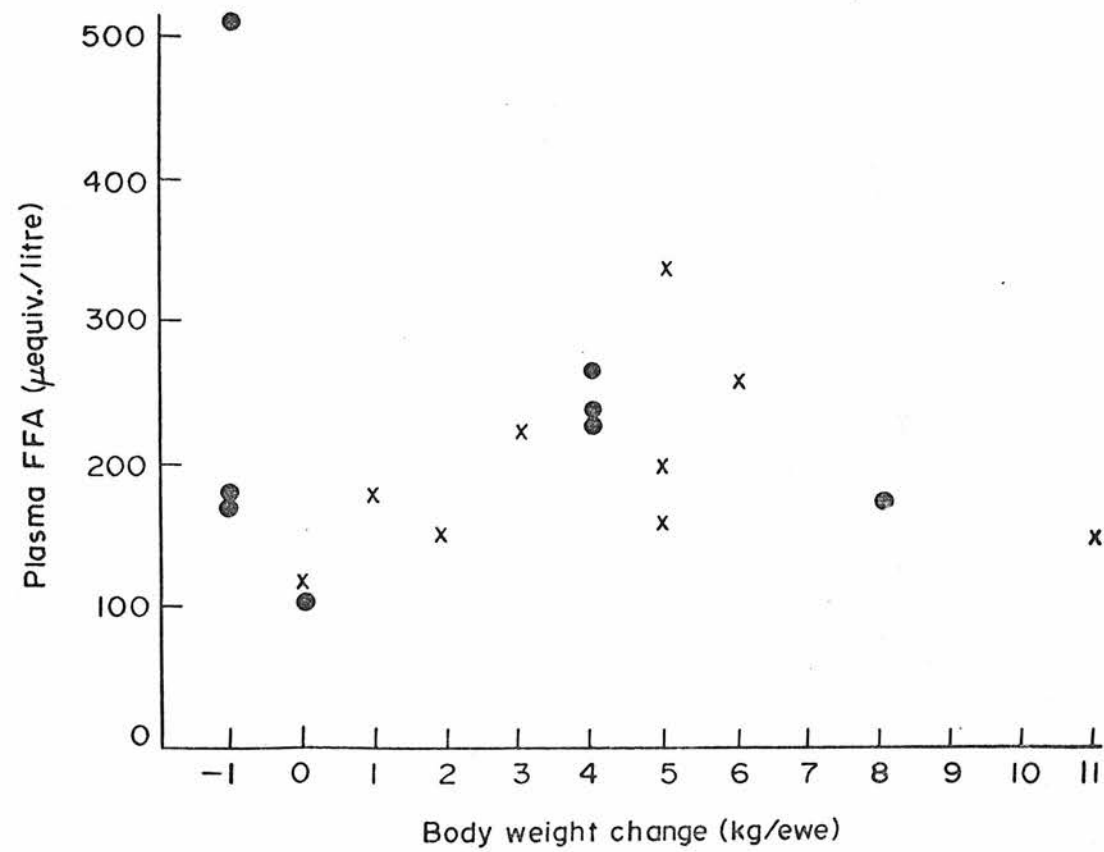
Table 4.2. Mean body weight gain and mean plasma FFA levels in the peripheral blood during the first 3 weeks after lambing for ewes which showed oestrus during this period compared with those which did not.

Group	Sub-group	No. of ewes	Mean body wt gain in kg/ewe \pm S.E. (range in parentheses)	Mean plasma FFA in μ equiv/litre \pm S.E. (range in parentheses)
Dry ewes*	Ewes in oestrus	8	2.1 \pm 1.2 (-1 to +8)	234 \pm 43 (105 to 510)
	Ewes not in oestrus	9	4.2 \pm 1.1 (0 to +11)	196 \pm 23 (115 to 337)
	Total	17	3.2 \pm 0.8 (-1 to +11)	214 \pm 23 (105 to 510)
Lactating†	Ewes in oestrus	1	0	427
	Ewes not in oestrus	32	3.0 \pm 0.6 (-3 to +12)	365 \pm 28 (88 to 631)
	Total	33	2.9 \pm 0.6 (-3 to +12)	364 \pm 26 (88 to 631)

FFA, free fatty acid * Lambs weaned 1 day after birth
 † Lambs weaned on the 24th or the 56th day of age.

Text-figure 4.1.

The relationship between body weight change and plasma FFA concentration for dry ewes which showed oestrus (●) or did not (x) during the first three weeks after lambing.



Synchronisation of oestrus 32 to 36 days post-partum

The timing of onset of oestrus following progestagen treatment is shown in Figure 4.2. All thirty-five ewes showed oestrus, and laparoscopy results (Table 4.3) indicated that thirty-one of thirty-three ewes had ovulated; the ovaries were not examined in two of the ewes. Although eleven of twelve ewes in the lactating group showed oestrus and ovulated following progestagen treatment, the interval from sponge removal to the onset of oestrus was 41 hours in these ewes compared with 34 hours in the dry ones ($P < 0.01$).

The pre-ovulatory release of LH

The LH release was characterised by a sharp increase in plasma LH concentration to more than 100 ng/ml in some ewes over a period of 2 to 4 hours with a rapid return to basal levels by 10 hours after the initial rise. The characteristics of the discharge in each of the three groups are given in Table 4.4, and illustrated in Figure 4.3, by representative animals from each treatment group. Only one of the thirty-five ewes failed to register a discharge, this being in the lactating group.

The interval between the onset of oestrus and the start of the LH discharge differed markedly between treatments (Table 4.4). This interval was only 9.1 and 6.1 hours in the non-lactating groups compared with 12.6 hours in the lactating groups ($P < 0.01$). But the baseline levels of LH in the three treatments were similar, ranging between 2.5 to 10.0 ng/ml plasma, as were the mean maximum values and the mean duration of the discharge (Table 4.4).

Text-figure 4.2.

The time interval between end of progestagen treatment (vaginal sponge withdrawal) and the onset of oestrus in ewes with 1 (●····●), 24 (●—●) or 56 (●——●) days of lactation.

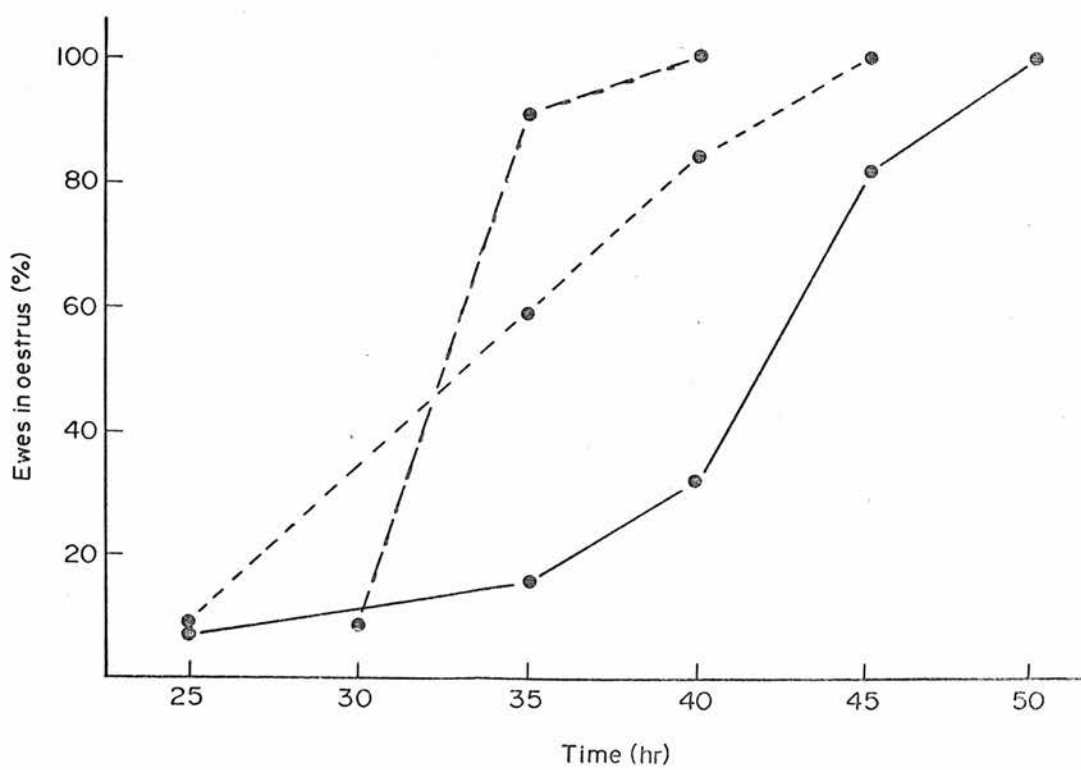


Table 4.4. The characteristics of the pre-ovulatory release of LH associated with synchronized oestrus 35 days post-partum for ewes with 1, 24 or 56 days of lactation.

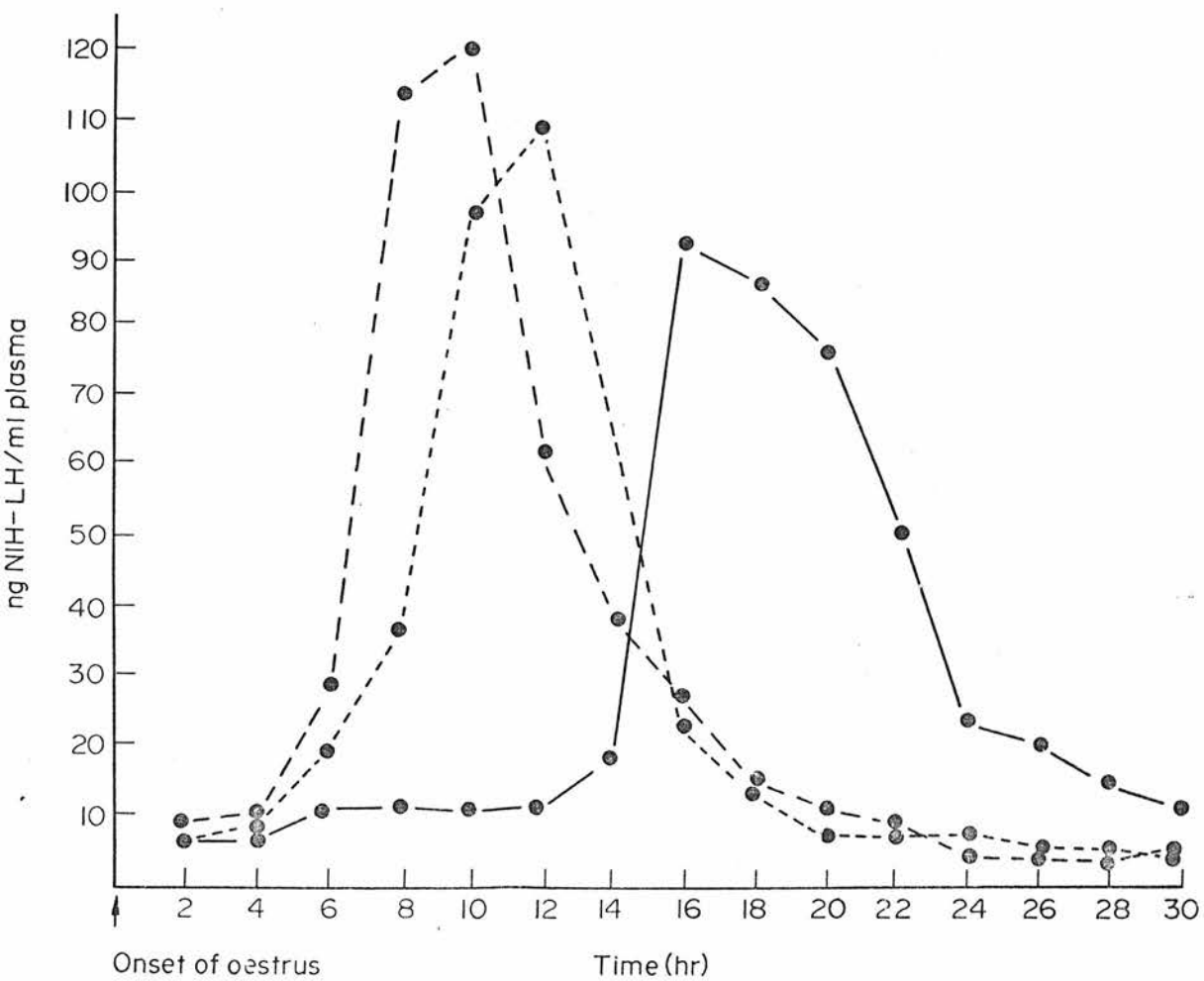
S Character	Duration of lactation ewes (days)	No. of	Extract from analysis of variance					
			Mean	S.E.	d.f.	M.S. between	M.S. within	F
Time interval between onset of oestrus and the start of LH release (hr)	1	12	9.09	1.21				
	24	11	6.09	1.27	33	119.0	17.6	6.8**
	56	11	12.64	1.27				
Maximum concentration of LH released (ng NIH-LH/ml)	1	12	102.3	12.5				
	24	11	125.3	13.0	33	1517	1872	0.8
	56	11	112.0	13.0				
Duration of the LH release (hr)	1	12	12.0	0.58				
	24	11	10.09	0.60	33	11.5	4.1	2.8
	56	11	10.55	0.60				
Integral of the area under the LH peaks (ng/ml/hr)	1	12	429	55				
	24	11	560	57	33	49170	35646	1.4
	56	11	493	57				

M.S., mean square; F, variance ratio

** P < 0.01.

Text-figure 4.3.

The pre-ovulatory LH release in three
ewes representing 1 (●---●), 24 (●—●)
or 56 (●——●) days of lactation.



DISCUSSION

The observation that only one of thirty-three lactating ewes, and eight of seventeen dry post-partum ewes showed oestrus during the first 3 weeks after lambing, indicates that the reproductive activity of the present flock of lactating sheep was almost completely suppressed following lambing in the middle of the breeding season (December), when they would normally be expected to show regular cyclical oestrus. This is in agreement with the report of Land (1971), that in two consecutive years lactating Finn-Dorset ewes did not show oestrus until approximately 45 days after winter lambing. Furthermore silent ovulations were not detected in any group during this study period, as observed in the cow (Short, Bellows, Moody and Howland 1972).

The reduced incidence of oestrus observed during lactation in well fed ewes was in accord with the conclusion of Restall (1971) that the presence of a lamb appeared to block the expression of a regular pattern of oestrus and to cause delay in the mean time of first ovulation, and also that of Mauléon and Dauzier (1965) who reported that whereas over 35% of Île de France ewes with lambs removed had their first heat before the 34th day after lambing, this occurred in only 10% of ewes that were suckling. By contrast, Hunter and Van Aarde (1973) concluded that if lactating and non-lactating ewes were fed to meet their respective nutritional requirements, the durations of their post-partum anoestr~~us~~ periods do not differ. The present results support the hypothesis that suckling delays the resumption of

reproductive activity following lambing in adequately fed ewes. The same conclusion has been reached for the cow (Short et al., 1972). The display of oestrus and the observation of ovulation in most of the ewes following treatment with a progestagen during lactation, however, indicates that such treatment may facilitate the resumption of ovarian activity.

A typical pre-ovulatory LH release similar to that found in cyclic ewes, and previously reported by several workers (e.g. Pelletier, Kann, Dolais and Rosselin, 1968; Mauer, Revenal, Johnson, Moyer, Hirata and White, 1972) was detected in the peripheral blood plasma of thirty-four out of thirty-five ewes. The time interval between the onset of oestrus and the release of LH in the lactating ewes, however, was longer than that previously reported.

The observation of a delay in both the onset of oestrus and the discharge of LH in the lactating ewes compared with dry ewes indicates that lactation does have an effect on LH release, as postulated in pigs (Crighton and Lamming, 1969), sheep (Land, 1971) and cows (England, Hauser and Casida, 1973) and as demonstrated in the rat (Hammons, Velasco and Rothchild, 1973) and in sheep during anoestrus (Pelletier and Thimonier, 1973). The difference in the display of oestrus and LH secretion for ewes which suckled for 24 and 56 days is compatible with the findings of Hammons et al. (1973). They reported a very rapid increase in the serum level of LH following removal of the litter, which indicates that the suckling stimulus must be applied

almost continuously in order to suppress LH secretion.

The effect of lactation on the timing of the onset of oestrus following sponge withdrawal and the release of LH is a factor which should possibly be considered in the choice of mating or insemination times for lactating sheep. The present failure to detect differences in the nutritional status of the lactating and dry ewes indicates that the differences in the incidence of oestrus before progestagen treatment, and the timing of events after treatment are not mediated by way of nutritional differences but are a direct effect of lactation.

CHAPTER 5

The Effect of Pre- and Post-Partum Feed intake on the Reproductive Activity of Suckling and non-Suckling Ewes

Introduction

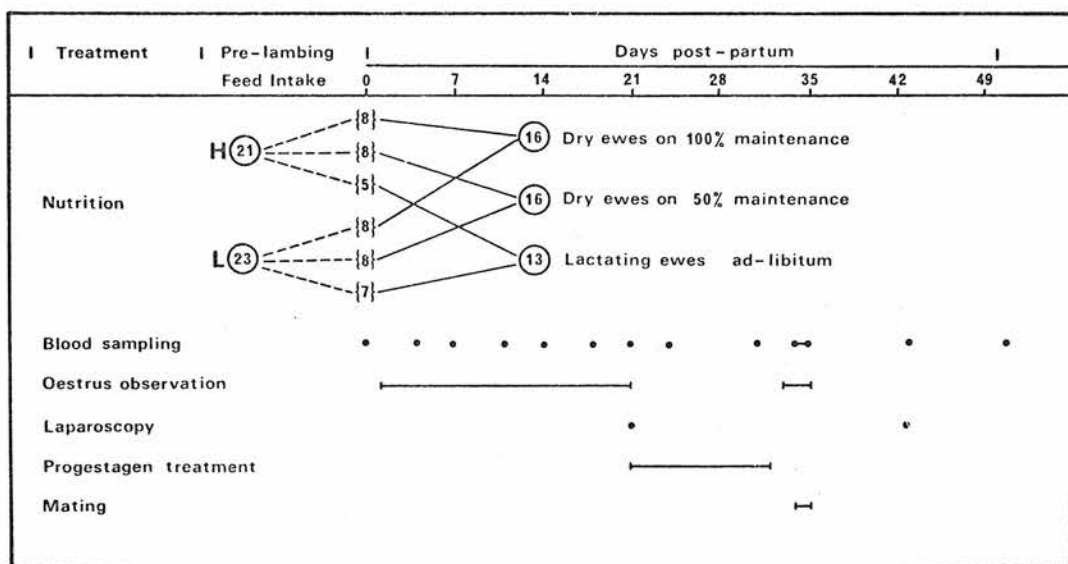
Nutrition is commonly associated with infertility, although the exact nature of the relationship is not fully understood. It has been assumed that the endocrinological control is affected, but it is not known whether the animals' secretory pattern of the pituitary gonadotrophins, or whether its reproductive function is affected by other factors concerning the target organs (for details see Literature Review, Sections B1. and B2.). In the present study, the effects of undernutrition on the ovarian activity, conception, and the plasma concentrations of progesterone, LH and prolactin were studied in post-partum lactating and non-lactating ewes.

Experimental

Forty-five ewes which were on 2 nutritional treatments, high (2H) and low (2L) energy intakes during the last 6 weeks of gestation as described in Chapter 1, lambed in mid-December, 1972, well within their breeding season (Land and McClelland, 1971). After lambing the ewes were divided into 3 groups (2 of 16 and 1 of 13) where the 2 pre-lambing treatments were equally represented. The two groups of 16 ewes each had their lambs weaned at one day of age, whereas the ewes of the third group suckled their lambs for 56 days (Text-fig. 5.1.). The dry ewes were housed in individual pens and the lactating ewes in a common pen with access to creep feed for the lambs.

Text-Figure 5.1.

Plan of the experiment and the number of
animals per group.



A complete ruminant diet (CRD) incorporating 40% straw and containing 1.8 Mcal ME/Kg and 9.6% digestible crude protein (DCP) was fed to the three groups at three different levels:

H - 16 dry ewes were fed a maintenance ration based on a recommended intake of 33 Kcal ME/Kg ewe live weight.

R - 16 dry ewes reduced to 50% of the above ration.

L - 13 lactating ewes, each suckling twin lambs were fed ad-libitum.

The nutritional state of the ewes was monitored by weekly body weight and blood metabolite determinations.

Plasma progesterone, LH and prolactin concentrations were determined in samples collected every 3 to 4 days during the first 3 weeks post-partum, on days 3 and 10 of the progestagen treatment, on the day of oestrus (every 2 hours) and Days 8 and 17 of the oestrus cycle after synchronisation.

Oestrus was detected by the introduction of vesectomised rams twice daily at 09.00 and 16.00 from Day 2 after lambing onwards. On Day 21 oestrus was synchronised by the insertion of intravaginal sponges inserted for 12 days (see Material and Methods). Twenty-two hours after sponge withdrawal, rams were introduced to the ewes for 15 minutes and then again at 2 hourly intervals for further 15 minute periods until oestrus was detected up to a limit of 50 hours after sponge withdrawal. During oestrus (Days 34 and 35) the ewes were mated to rams known to be fertile on two occasions, 12 hours apart, individually or in small groups.

Ovulation was studied by counting the number of CL on each ovary using a laparoscope under general anaesthesia. Twenty-four ewes (8 of each treatment) were studied on Day 21 and all the 45 ewes 8 days after the synchronisation of oestrus.

RESULTS

The effects of the nutrition treatments are summarised in Table 5.1. and Text-figure 5.2. The pre-lambing feed intake had no effect on the nutritional state or the reproductive activity during the post-partum period and therefore the two subgroups were pooled within each post-partum treatment. The energy intake of 33 Kcal ME/Kg ewe live weight, fed to the H group was found to be adequate to meet the nutritional requirements for maintenance. The ewe live weight was relatively stable after short fluctuations, and the weekly determinations of the blood metabolites; glucose, FFA and ketone bodies indicated that the concentrations of these metabolites were all within the normal range (Patterson, 1963). Undernutrition in the R group was indicated by a continuous decline in body weight (about 1 Kg/ewe/week) and an increase in plasma FFA up to 650 μ equiv/litre, by the third week of the experiment. The ad-libitum fed lactating ewes consumed an average of 6 Mcal ME/ewe/day and were able to maintain body condition at the initial level for the early part of the experiment, but failed to do so towards the end. In the last 2 weeks there was a marked drop in the mean live weight and the plasma concentrations of FFA and ketone bodies reached the values of 500 μ equiv/litre and 3 mg% respectively,

Table 5.1. Body weight change, feed intake and peripheral blood metabolite concentrations during the first 50 days post-partum for suckling and dry ewes on various feed levels. Overall means were calculated from the weekly means.

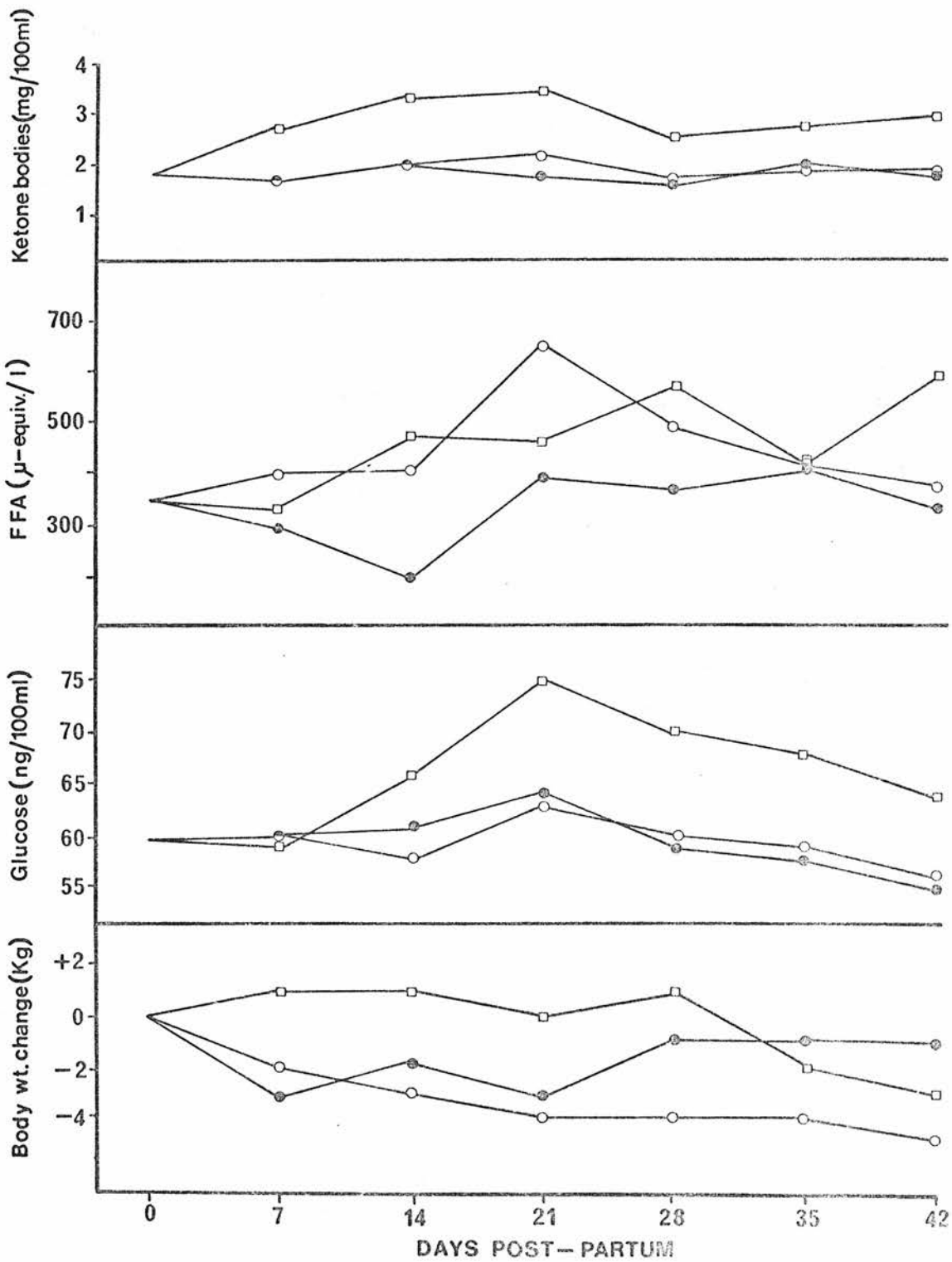
Treatment	No. of ewes	Body wt at parturition (Kg)	Daily feed intake		Body wt change (Kg)	Plasma glucose (mg/100 ml)	Plasma FFA (μ equiv/litre)	Plasma ketone bodies (mg/100 ml)							
			Energy (Mcal ME/ewe)	Protein (g DCP/ewe)											
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
H Dry ewes 100% Maintenance	16	64	1.8	2.1	0.04	120	1.6	-1.13	0.84	60	0.9	324	22	1.8	0.1
R Dry ewes 50% Maintenance	16	59	1.8	1.0	0.04	60	1.6	-4.50	0.84	59	0.9	445	22	1.9	0.1
L Lactating ewes <u>ad-libitum</u>	13	66	1.9	6.0	0.05	320	1.8	-2.20	0.93	66	1.0	459	24	2.8	0.1

ME, metabolizable energy; DCP, digestible crude protein; FFA, free fatty acids

Analysis of variance - * = P < 0.05; ** = P < 0.01.

Text-Figure 5.2.

Body weight change and the peripheral plasma concentrations of glucose, free fatty acids (FFA) and ketone bodies measured at weekly intervals during the first 6 weeks after lambing in suckling ewes fed ad-libitum (□) and non-suckling ewes fed either 100% (●) or 50% (○) of the maintenance requirements.



both of which indicate the start of undernutrition in the ewe.

Reproductive activity during the first three weeks post-partum.

The number of ewes which exhibited oestrus and ovulated during the first three weeks post-partum is given in Table 5.2. Feed restriction to 50% of the maintenance requirements to dry ewes (R), led to a slight but statistically non-significant reduction in the number of ewes showing oestrus and delayed the day of onset; 8 of 16 R ewes showed oestrus between 8 to 19 days post-partum (mean interval 16.9 days), compared with 11 of 16 H ewes between 10 to 20 days (mean interval 15.5 days). The ovulation and the ovulation rate of those ewes which showed oestrus was not affected by the nutrition treatment, as was verified by the number of CL observed by laparoscope on Day 21 (Table 5.2). Lactation did however inhibit oestrus and ovulation during this period. Only one of 13 lactating ewes exhibited oestrus and ovulated during the first three weeks post-partum.

The plasma concentrations of progesterone and LH measured every 3 to 4 days and prolactin measured once a week, are illustrated in Text-figure 5.3., and their overall means for the three week period are given in Table 5.3. Plasma progesterone was found to increase with time from the very low levels (0.26 ng/ml) measured at parturition to higher average levels in the dry ewes (H and R) but no increase was observed in the lactating ewes. The plasma LH values fluctuated and varied between and within treatments and no trend of change could be seen in the basal LH levels. In

Table 5.2. Ovarian activity before and after synchronization of oestrus 35 days post-partum in dry ewes on high and low levels of nutrition and in ad-libitum fed lactating ewes

Treatment	No. of ewes	2 to 21 days after lambing		After sponge withdrawal				Lambing rate				
		No. of ewes in oestrus	No. of ewes ovulated/ oestrus ewe examined+	Mean No. of CL	Onset of oestrus No. of hrs. after ewes withdrawal	No. of CL observed on day 8 of the cycle	No. of ewes conceived ++					
				Mean	SE	Sig	Mean	SE	Sig	No.	%	
Dry ewes	16	11	5/8	2.2	13	35.5	1.9	2.14	0.19	12	6	38
H 100% maintenance												
Dry ewes	16	8	5/7	2.8	15	36.3	1.7 (NS)	2.07	0.19 *	11	1	6
R 50% maintenance												
Lactating ewes	13	1	1/8	3.0	11	39.3	2.0	2.80	0.21	9	5	39
L ad-libitum												

+ all the ewes examined in treatments H and R had shown oestrus. In treatment L, only the one ewe which ovulated had shown oestrus.

++ conception was assumed to take place in ewes which had high levels of progesterone on the last day of synchronized cycle (Day 17).

Analysis of variance * = $P < 0.05$.

some cases very high levels were measured when blood samples were taken on the day of oestrus. The display of oestrus in groups H and R was reflected by the overall means of progesterone and LH that were higher than those of the lactating ewes. Although the differences between the three groups were not statistically significant ($P > 0.05$), the specific comparison of dry and lactating ewes indicated that the concentration in the dry ewes was greater than that of those lactating ($P < 0.05$).

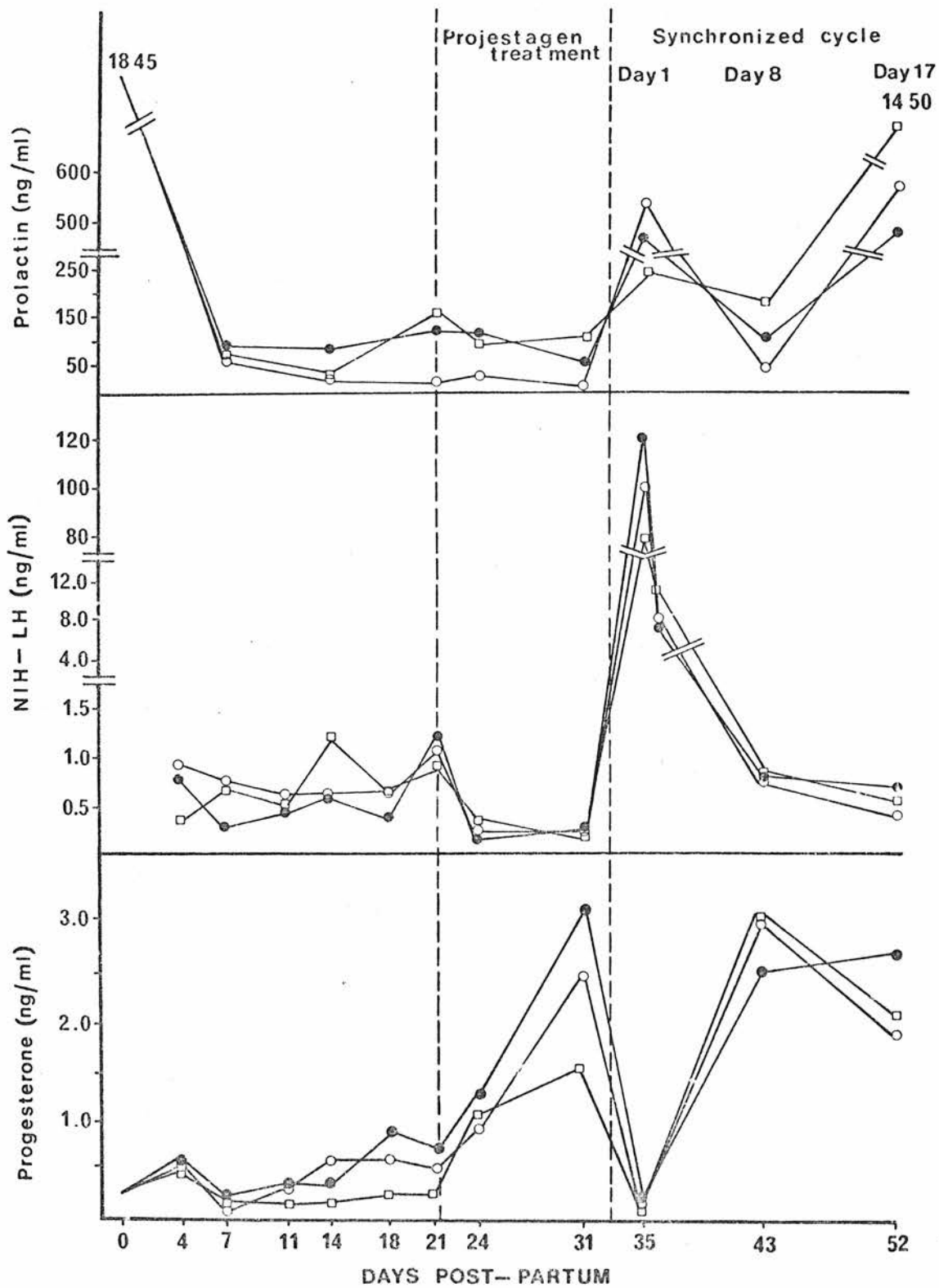
After the high prolactin concentrations around the time of parturition, the plasma prolactin levels were low during the post-partum period. They seem to be affected by the nutritional level, being higher in the non-lactating and high fed ewes than in those on reduced diet. Considerable variation was however found between individuals within treatments, and the differences between the means were not significant ($P > 0.05$).

The effects of the progestagen treatment given from Days 21 to 33 post-partum.

Insertion of progestagen impregnated vaginal sponges, gradually increased the plasma levels of progesterone in the ewes. This increase was much higher in ewes of treatment H than in those of R and L treatments. The plasma progesterone levels in dry ewes (H) increased from 1.3 ng/ml on the third day of the treatment to 3.2 ng/ml on Day 10. In the three treatments the rise in plasma progesterone seems to be related to the existing levels before the ewes were treated with the progestagen (Text-figure 5.3.)

Text-Figure 5.3.

Peripheral plasma levels of progesterone, luteinizing hormone (LH) and prolactin during the first 3 weeks post-partum, Days 3 and 10 of the progestagen treatment and Days 1, 8 and 17 of the oestrous cycle after synchronization (Days 35, 43 and 52 post-partum), in suckling ewes fed ad-libitum (□) and non-suckling ewes fed either 100% (●) or 50% (○) of the maintenance requirements.



The LH levels dropped during the progestagen treatment to about 0.2 ng/ml with negligible differences between treatments.

No effect on the prolactin secretion was evident during the progestagen treatments.

The progestagen treatment was fully effective in suppressing oestrus in all the ewes during the treatment. Following sponge withdrawal on Day 33, oestrus was observed in 39/45 ewes on Days 34 or 35 post-partum. Nutrition and lactation treatments had no effect on the number of ewes showing oestrus, but there was an indication that the interval from sponge withdrawal to onset of oestrus was longer in the lactating ewes (Table 5.2). The ovulation rate was also higher in the lactating ewes (2.8) than in the dry ewes (2.1) ($P < 0.05$).

Plasma hormone levels during the synchronised oestrous cycle

Blood samples were collected at 2 hourly intervals during the day of oestrus to study differences in the pre-ovulatory LH release between the three treatments. Table 5.3 summarises the differences in the time interval from onset of oestrus to the start of LH discharge, the maximum concentration and the time duration of the LH secretion. Although the start of LH release was delayed (up to 22 hours in one case) and the maximum LH concentrations were lower in the lactating ewes, the only parameter to be significantly affected by lactation was the duration of the discharge. This was shorter in the lactating ewes. No effects of under-nutrition on the secretory pattern of the pre-ovulatory LH

Table 5.3. Plasma concentrations of progesterone, LH and prolactin in Days 1 to 21 after lambing, and the characteristics of the pre-ovulatory release of LH at synchronized oestrus 35 days post-partum for dry ewes on high and low levels of nutrition and ad-libitum fed lactating ewes.

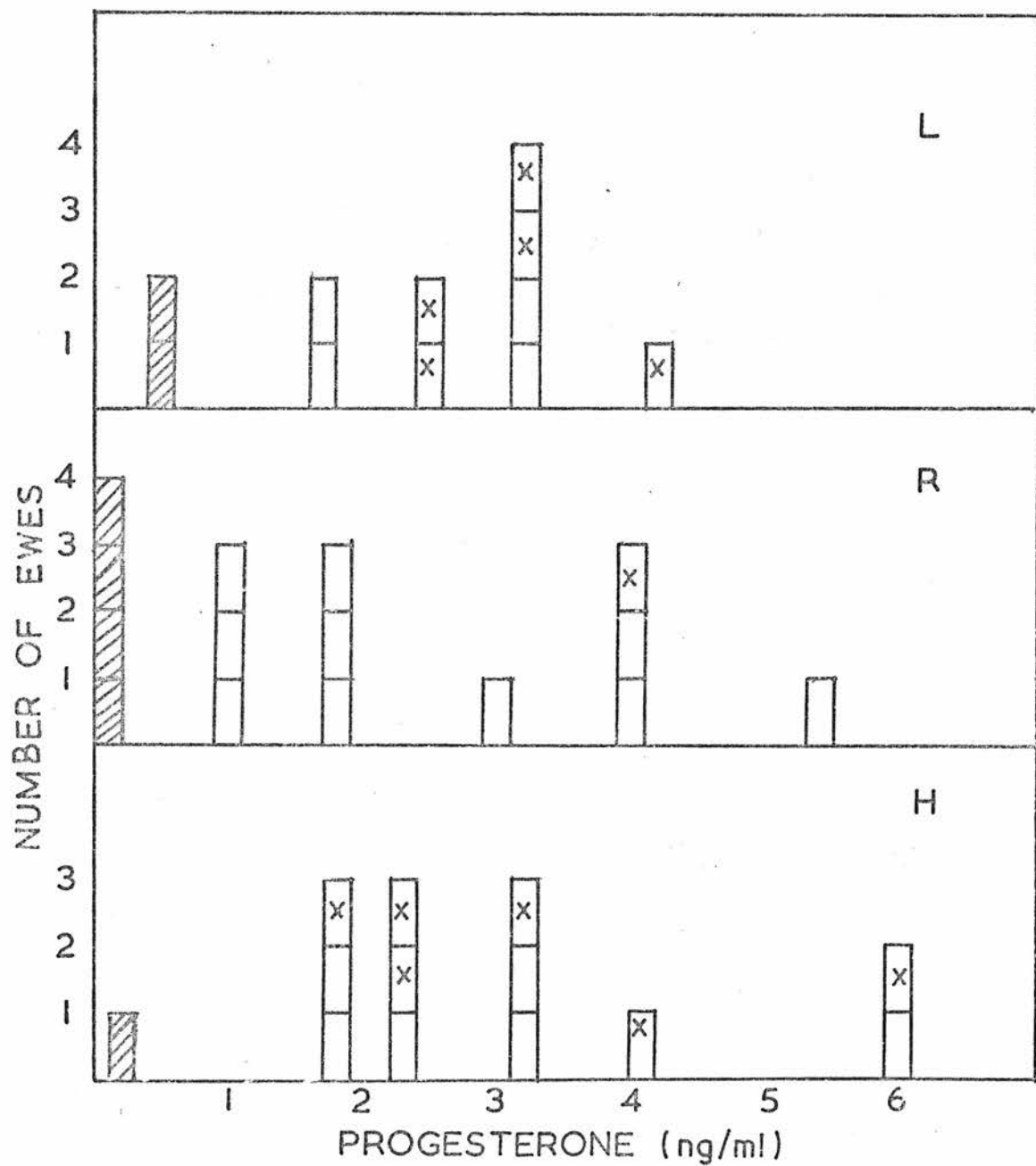
Treatment	No. of ewes	Progesterone (ng/ml)	LH (ng/ml)	Prolactin (ng/ml)	Start of LH release after onset of oestrus (hrs)	Maximum conc. of LH released (ng/ml)	Duration of the LH release (hours)
		Mean SE Sig	Mean SE Sig	Mean SE Sig	Mean SE Sig	Mean SE Sig	Mean SE Sig
Dry ewes 100% main-tenance	16	0.50 0.09	1.78 0.44	102 43	4.8 1.2	118 12	12.6 1.0
Dry ewes 50% main-tenance	16	0.44 0.09(NS)	1.45 0.44(NS)	29 43(NS)	5.5 1.2(NS)	96 12(NS)	13.5 1.0 *
Lactating ewes <u>ad-libitum</u>	13	0.22 0.10 *	1.04 0.48 (NS)	80 44 (NS)	8.0 1.3 (NS)	82 14 (NS)	9.3 1.1 *
Total dry ewes	32	0.47 0.06	1.61 0.31	66 30	5.1 0.8	107 9	13.1 0.7

Analysis of variance * = $P < 0.05$

Text-Figure 5.4.

Frequency distribution of plasma progesterone concentration, 17 days after mating, 35 days post-partum for dry ewes on high (H) and reduced (R) intake and lactating ewes (L) fed ad-libitum.

(the crossed columns indicate ewes not considered to be pregnant: X indicates ewes which lambed)



were evident in the dry ewes.

The plasma LH values measured in latter dates of the cycle were very low (0.4 to 0.7 ng/ml) and did not differ significantly between the various treatments or between days (Days 8 and 17) of the oestrous cycle.

Plasma prolactin levels were found to be high on the day of oestrus, declined in mid-cycle and increased again by the end of the oestrous cycle (Text-figure 5.3). The increase in plasma prolactin on Day 17 was associated with low levels of plasma progesterone in the dry ewes. Ewes which had a high progesterone level on Day 17 had in most cases prolactin levels similar to those measured in mid-cycle. In the lactating ewes the effect of suckling on prolactin secretion obscured these changes.

Fertility

The ewes were mated with fertile rams during the synchronised oestrus. The conception rate as predicted from the progesterone levels 17 days after mating averaged 87% for all treatments (ewes which maintained high progesterone concentration i.e. > 1 ng/ml were considered to have conceived (Text-figure 5.4)). However, the lambing data at the end of gestation (Table 5.2) showed that fertility was markedly affected by the feed intake after the previous lambing; only one of 15 ewes showing oestrus in treatment R lambed (1 single lamb) compared with 6/13 and 5/11 ewes from H and L treatments respectively, which lambed an average of 1.7 lambs each.

Both dry ewes on maintenance ration and lactating ewes

on ad-libitum feeding, showed relatively low fertility (38.5% for both groups) when mated after a progestagen treatment 35 days post-partum within their breeding season.

DISCUSSION

The display of oestrus by only one of 13 lactating ewes compared with 11 of 16 dry ewes, despite the adequate nutrition of both groups, supports the hypothesis that suckling has a direct effect on the resumption of reproductive activity following parturition in adequately fed sheep (Chapter 4), rather than an indirect effect via nutrition. Ovarian activity was only slightly affected by a reduction in the diet of non-lactating ewes.

The presence of CL observed in dry but not in lactating ewes, was associated with high concentrations of progesterone and possibly LH in the peripheral plasma during the first three weeks after lambing. The concentrations of these two hormones were not affected by the state of undernutrition imposed on the dry ewes in treatment R, but remained similar to those of the well fed H group throughout the period of study. Despite this similarity in hormone levels observed in the fully fed and restricted groups around the time of mating, however, only one of 16 restricted ewes carried a lamb to term, compared to 6/16 fully fed ewes. In sheep, therefore, the present data indicate that the influence of reduced feeding on fertility is not solely through variation in the particular hormones measured in the present experiment, around the time of mating.

The low fertility (38.5%) found in the well fed lactating and non-lactating ewes (H and L) is consistent with previous findings that fertility to natural and artificial matings at synchronized oestrus is lower than normal (Fletcher, 1971; Roche and Crowley, 1973), although McClelland and Quirke (1971) did obtain good results in Fimm-Dorset ewes. The main cause of this low fertility was thought to be a failure of fertilisation (Moore, Quinlivan, Robinson and Smith, 1967), but the present data and that of Gunn, Doney and Russel (1972) point out that foetal death might also be an important factor, but this may limit the usefulness of progesterone determination on Day 17 after mating for early pregnancy diagnosis as suggested by Shemesh, Ayalone and Lindner (1968).

No relationship was found between progesterone concentration and the number of CL produced, supporting the suggestion of Lamond and Gaddy (1972), that naturally polytocous species maintaining blood levels of progesterone within defined limits regardless of the actual number of CL formed. Similarly, the concentration of progesterone was not found to be related to that of LH, an observation consistent with the finding that young CL were not sensitive to LH stimulation (Land, Collett and Baird, 1974), and are probably secreting maximally.

The delay in the onset of oestrus and the longer interval between the onset of oestrus and the release of LH, and the shorter duration of the discharge, although not all statistically significant are consistent with early results

of Pelletier and Thimonier (1973) and the results reported in Chapter 4. Also the relationship between these intervals and the ovulation rate is consistent with the between breed findings of Land, Pelletier, Thimonier and Mauléon (1973).

GENERAL DISCUSSION AND CONCLUSIONS

The increasing demands on the utilisation of land for urban and recreational purposes and the resulting increase in land value are likely to force a re-appraisal of the relative economic performance of suitable agricultural enterprises. The sheep as a grazing animal at its present level of productivity is generally inferior both biologically and economically to such alternatives as dairy cattle or crop production. Hence there is the possibility that sheep production will be restricted to land areas which are unsuitable for reasons of climate or topography for urban and other agricultural use. To avoid this, the possibility exists for the future of the industry to develop an intensive housing management system within which sheep having high reproductive potential, high growth rate and good food conversion will be maintained.

Under most present situations ewes are producing lambs on only a part-time basis, although enough information is available for some producers, at least, to increase the efficiency of lamb production to a point where it can compete with any other form of livestock production. An increase in the number of lambs born per unit of time can be achieved by an increase in the average litter size, increased frequency of lambing, or by a combination of these two. Both can be considerably influenced by management.

In the search for intensification, there could be two approaches: 1. Intensive ranching at one extreme, on the land area which is obviously not capable of becoming arable land

and 2. the other extreme, of intensive indoor production in which sheep would be farmed like poultry or pigs. These systems of production will require the sheep to step from seasonal to all the year round breeding and to introduce the techniques forced upon the poultry and pig industry.

For these changes to take place, new problems have to be overcome and new expertise has to be developed. In the following, attempts will be made to discuss the needs of an intensive sheep production system aiming at two crops of lambs a year.

Type of sheep

In the intensive systems of production the aim will be to evolve animals which will breed regularly out of the normal breeding season, will be highly prolific and will give birth to rapidly maturing lambs. To obtain two lamb crops a year it is necessary to use animals with extended breeding seasons and shortened post-partum and lactation anoestrous. Several breeds exhibit long breeding seasons, naturally, and selection for this characteristic could be carried out although it is not clear how strongly this is inherited. Litter size could also be raised considerably, both by genetic and management improvements above the generally accepted level of 1 to 2 lambs per year.

Sheep production depends to a considerable extent on crossbreeding. Since heterosis is most likely to occur between breeds the less closely they are related, the derivation of breeds is of considerable importance. The present results could be interpreted to indicate an advantage

in favour of crossbreds as against purebreds. The Finn-Dorset (F_1) ewes used in this study combined a high incidence of multiple births with a prolonged breeding season. These sheep, under appropriate conditions, are capable of lambing regularly at 6 to 7 month intervals, although the need for stimulating post-partum oestrus by using exogenous hormones seems to be necessary.

Hormone treatment

Ewes on a true twice a year lambing schedule must be rebred within 40 days after lambing. This requires breeding during a period when the ewes are still experiencing post-partum and lactation anoestrus and in some individuals, seasonal anoestrus as well. Each breeding must therefore be artificially induced, even when occurring during the usual breeding season, because of the influence of post-partum and lactation anoestrus. On the other hand there is no clear requirement for early weaning to promote the occurrence of early post-partum heat. Synchronisation of oestrus following progestagen treatment from the 21st day post-partum for 12 days was successful in almost all the ewes treated whether lactating or not. However, both the onset of oestrus and the discharge of LH were delayed in lactating ewes compared with dry ewes. The late occurrence of oestrus and the LH release in lactating ewes are factors which should be considered in choice of mating or insemination time for lactating sheep.

It is not clear whether the level of nutrition of ewes can greatly influence the return to heat after lambing. In the present study, there was ^{little} ~~no major~~ indication of inter-

action between the effect of lactation and feeding level. In 2 consecutive years, lactating ewes on ad-libitum feeding consumed 6 to 7 Mcal ME/ewe/day and their state of nutrition was considered sufficient to meet their lactation requirement. Despite adequate nutrition, the oestrus display and ovulation of lactating ewes ~~were~~ almost completely suppressed following lambing in the middle of the breeding season, when they would normally be expected to show regular cyclical activity. This finding, together with the observation that feed restriction to 50% of the maintenance requirement of dry ewes caused only a slight and non-significant reduction in the number of ewes showing oestrus, with no effect on ovulation and ovulation rate, led to the conclusion that suckling has a direct effect on the resumption of reproductive activity following parturition.

Nutrition of the pregnant ewe

The subject of most interest and importance in intensification is that of nutrition of the pregnant ewe, especially in the case of multiple pregnancy where metabolic disorders may result from the enhanced stress of greater prolificacy. Pregnancy toxæmia which is associated with undernutrition of the ewe during the last 6 to 8 weeks before lambing is likely to be important in intensive systems. The ewe should be fed to suit the requirements for multiple foetus development. A need clearly exists for a quick, cheap and reliable means of determining both multiple births and the nutritional status of the ewe.

Laparotomy is the only procedure available at the

present time for detection of pregnancy within the first trimester. Diagnostic ultrasound can be used during the second and third trimesters of pregnancy. Positive Doppler responses give absolute assurance of pregnancy and that the foetus is alive. Echo ultrasound techniques may be used with external or internal probes and have been shown to be very useful for rapid screening procedures but with less accuracy. Preliminary work indicates that it may be possible to develop a laboratory test of pregnancy in the ewe based on agglutination-inhibition reactions.

In the present study X-ray examination at 90 days of gestation was satisfactory. It was possible in this way to identify foetal bones and to count the number of foetuses with high accuracy. But, it can hardly be regarded as a technique capable of wide practical application.

More progress has been made toward the use of plasma and milk progesterone for pregnancy diagnosis. Conception can be predicted from the progesterone levels 17 days after mating, although high embryonic mortality may limit the use of early pregnancy diagnosis. The number of lambs carried by the ewe ~~can~~^{may} be predicted from the plasma progesterone concentration measured around the day 100 of gestation. However, undernutrition was found to cause a greater increase in progesterone concentration than did the extra lamb. Hence, the use of peripheral progesterone concentration to determine twinning in a flock situation would probably be complicated by variations in the nutritional status of the dam. A relationship between plasma progesterone concentration and foetal weight was also reported and this is another

factor which might influence any such comparisons. Inevitably there is going to be a wide range of foetal weight variation within a flock under field conditions.

In the present study, of the criteria used to assess the nutritional state of the ewe, the FFA concentration in the peripheral blood plasma as an indicator of energy metabolism appears to be most sensitive in demonstrating differences in energy intake even between treatments where high energy intakes were employed. This confirmed the FFA as a valuable index of nutritional state although the various contents of glycogenic material in various diets might have an effect on the concentrations. A significant relationship was observed between plasma FFA and plasma progesterone concentrations of pregnant ewe, thus showing a possible effect of nutrition on the maternal plasma progesterone concentration. This raises the possibility of future use of plasma progesterone concentrations as a criterion for the assessment of the nutritional state in sheep. Body weight changes were found to be a crude measure and failed to replace short term nutritional differences as it can be seen in Experiment 1, part 1, where there was no significant difference in the body weight change between treatments, although energy intake varies between 3.4 to 4.4 Mcal ME/ewe/day. This occurred even though the effect of the changing foetal weight on the maternal weight was avoided by adjusting feed intake within treatments according to litter size. There were also minor differences between the treatment groups in terms of lambs born, total lamb birth weight and litter size.

High energy intake, in late pregnancy, on the one hand and restricted on the other, seems to have very little effect on the lamb birth weight thus confirming the limitation of use of the lamb weight as an index of adequacy of nutrition in late pregnancy as discussed by Russel et al. (1967a).

Based on the present findings it was concluded that a 65 Kg ewe bearing twin lambs with no change in body weight in the early stages of pregnancy, will require about 4 Mcal ME/day during the last 50 days of gestation to prevent a rise in plasma FFA concentration or loss of weight over gestation.

The inclusion of roughages in a mixture with concentrates fed in this study could result in a reduction in the digestibility of the fibre fraction of roughages. Also, the physical reduction of particle size of a roughage might increase the speed with which it leaves the rumen, thus allowing less time for microbial attack of the fibre while on the other hand, the increased rate of passage would probably stimulate intake. This greater intake, even with the reduction of digestibility of the fibres will result in greater overall performance.

Induction of parturition

The length of gestation will be of significance in intensive sheep production systems. To control the natural variation in gestation length and to synchronise parturition to make the whole lambing period shorter could have major advantages. In the present study artificial stimulation of parturition by an injection of dexamethasone on 141st day of

gestation, resulted in parturition within 49 hours after injection with no ill effect to the ewe or the lambs born. The parturition was preceded by declining maternal progesterone levels to well below 1 ng/ml on the day of parturition as in normal delivery and high and low feed intake during late pregnancy did not have any effect on the events associated with the initiation of the induced parturition.

Fertility

Production efficiency can be lost by either too much or too little feeding. Over-feeding produces a large fat animal which requires more feed to maintain the higher body weight. There are indications, in the phase during which the fertilized egg establishes itself as a developing foetus, that over-fatness may be a disadvantage and results in foetal atrophy. Since there is also evidence that poor feeding during mating and early gestation can lead to foetal loss and lower lambing rate, there would appear to be grounds for the generally held view that body weight should be maintained or slightly increased during the mating period. This view was strongly supported by the results of the present study.

The present data, however, indicated also that even in cases of adequate nutrition before mating, lambing rate after mating at a synchronised oestrus 35 days post-partum is only approximately 50% of ewes mated. This low fertility is consistent with previous findings that the fertility to natural and artificial matings at synchronised oestrus is lower than normal (Fletcher, 1971; Roche and Crowley, 1973).

It is not immediately apparent what precisely adversely affects fertility. It is possible, that inadequate synchronisation of ovarian and uterine activity could be responsible. Apparently the uterus involutes quite rapidly. Within 2 weeks after parturition it is of normal size. But it is possible that this involution is actually not a complete normal involution in the sense that the uterus has recovered its ability to provide the proper environment for the lamb. In the present study conception rate, as predicted from the plasma progesterone concentration on Day 17 after mating, was over 80%. Therefore factors such as semen quality, sperm transport and altered cervix mucus following intravaginal sponge treatment could not be implicated as contributing to the low fertility. The early embryonic loss could be related to a possible steroid hormone insufficiency or imbalance. However, whether exogenous hormone supplementation will reduce embryonic mortality is still unclear. Preparation of the uterine environment by inducing one or two cycles in order that the ewes are in fact mated at the first repeated (spontaneously occurring) heats was suggested as a method for overcoming the relative infertility, but there is still the possibility that a proportion of the ewes may not recycle.

Good management of rams with which ewes were then either bred to each of several rams or 'hand bred' to rams of high fertility was reported to result in conception rates as high as 80% (Tweed, 1969; Jennings and Crowley, 1969; McClelland and Quirke, 1971). It is therefore essential to consider the ram when planning an accelerated lambing

system. It is fairly certain that fertility and libido in the ram are often lower in the period of ewe anoestrus than in the normal breeding season, and also that fertility is likely to fail long before libido in many rams. Apart from inherent semen fertility differences between rams, fertility differences may also result from mating preferences and other mating behaviour. These in fact are some of the arguments in favour of using artificial insemination as a means of overcoming wide variations in the number of sperm available for fertilisation. Artificial insemination could be a desirable means of controlling the effect of the ram in out of season breeding systems. However, for artificial insemination to be considered as a practical alternative to natural service there is the need to gain precise control of ovulation in the progestagen treated ewe, so that insemination can be performed at a standard time and not be dependent on prior detection of behavioural oestrus. Also, the problems associated with storage of ram semen must be solved.

Management

Housing - The sheep production system being discussed would require the housing of the animals for part or most of their lives, providing facilities for early weaning and artificial rearing of surplus lambs. The problems of waste and effluent disposal must then be considered. Provision of adequate shelter and lambing pens could greatly reduce, if not eliminate, the existing wastage of new born lambs,

Hygiene - In an intensive system health problems are likely to be one of the major considerations which has

been the case with poultry and is becoming increasingly so with pigs. The more animals that are brought together in one environment and under a system calculated to make the maximum use of a minimum of labour, the more favourable is the situation for the spread of infectious and contagious diseases. Also, the success of intensive systems is related to the limits to which the animal's metabolic capacity can be pushed. This pressure is such that metabolic and stress disorders tend to be more common. Health hazards can, however, be foreseen, and preventive measures can be adopted. It must be realized that it is basically in the area of environmental and nutritional conditions that the foundations of health in such flocks are laid.

Economic factors - A general intensification of sheep production will require greater management skill and more technical knowledge than has been previously employed. The largest single cost item in the ewe flock budget is feed. Therefore the development of an efficient management programme will involve an understanding of the nutritional requirements of the ewe and lambs at certain critical stages of their performance. The supply of milk substitute may be an important component of systems leading to high lamb productivity. Full recording and proper means of identification and reassessment of performance will be extremely important. Also improved management could reduce the early mortality of lambs to 2 or 3%, which has been achieved with other species.

Capital costs are likely to be higher for lambs produced in accelerated programmes, because some of the lambs must be produced during less favourable seasons and the system may also involve early weaning for some animals. Thus, producers' attitudes towards such programmes may well vary with the price of lambs at slaughter. In such a programme the overhead costs of the first 2 years of the ewe's life becomes less important if more lamb crops per year are produced. Also the present relatively high cost of the hormone treatments need not be so significant in future if increasing demands affect the economics of scale in their production.

The proposed twice a year lambing system has not been fully evaluated in economic terms, as a specific sheep management programme for general application is not fully realistic, because of considerable variation of the economic resources among producing units. However, there is already much experimental information to indicate that frequent breeding sheep systems can be carried through successfully. The determining factor as to how much of it will be practised in the future will be the economics involved. This in turn will be influenced by the productivity of the sheep.

Summary

Controlled breeding, quite apart from the specific practical advantages it may have to offer, could bring an important change in thinking in sheep production. Systems can be planned in which regular management tasks are performed according to a precise calendar arranged months in advance.

Satisfactory methods of hormone-controlled breeding will bring substantial benefits to the sheep industry, e.g., increased lambing frequency, increased litter size and the availability of a crop of lambs at any pre-determined time throughout the year. A more compact lambing season is also an advantage together with the accompanying benefits of more efficient production and marketing, and extensive use of artificial insemination, associated also with the full use of rams of outstanding breeding merit.

Based on these developments, intensive sheep production systems can be established in which large numbers of animals are housed throughout their lives in sophisticated buildings and bred as frequently as possible. Such systems would require a highly prolific breed that can breed 'out of season' and a highly qualified staff with knowledge of various aspects of production.

The present discussion has so far concentrated on the biological aspects, but other important inputs have only been touched upon to a limited extent. There is much that remains to be done in translating the knowledge which is presently available into practical economic realities and in transmitting requirements to farmers. It cannot be said yet if this method will prove the most economic, but it represents one of the main possibilities of intensification of the process of sheep production, as the sheep in future may well in many situations not be a grazing animal.

FUTURE RESEARCH

At the end of our short programme of research described in this thesis it is of value to outline the nature of further research programmes aimed at improving sheep productivity.

Comprehensive description of experimental programmes cannot be undertaken without considerable detailed data in the particular problem to be examined. However, the following areas would justify increased research activity.

1. The results reported in this thesis were obtained in environments not as extreme as the environments presently occupied by sheep and further research to convert into commercial practice the potential for induction of oestrus and fertile matings of ewes by hormonal control is needed, particularly in view of the required standard of management and the type of environment in which sheep are likely to be kept in the future.
2. Examining further exotic breeds of sheep as a potential means of obtaining a source of genes for less seasonal restriction of breeding and a higher reproductive rate in general.
3. Research into the lifetime response of sheep to different levels of nutrition may be able to define the optimum size and weight of sheep relative to their genetic potential and the critical periods of growth or critical seasons of ^{the} year with regard to total feed requirements, especially as they apply to reproduction.

It seems logical that the nutrition of ewe lambs from birth to the production of the first lamb could be very critical in relation to lifetime potential for multiple ovulation, but research data is lacking on this point.

4. In intensive production systems there is the possibility that large litters or more frequent breeding might reduce the longevity of the female or might increase the natural mortality rate, thus decreasing mean longevity due to the greater total stress of pregnancy. There is no evidence of this as yet.
5. For the successful exploitation of control breeding, it is necessary to obtain a satisfactory conception rate at the controlled oestrus ; hence the factors responsible for the sub-fertility encountered at the first oestrus following hormone treatments deserve immediate and serious study. We lack also adequate basic information on the total effects of administered hormones, interactions of hormone action and external environment.
6. Evidence has accumulated to indicate that luteal regression in the sheep is initiated by the production of a luteolytic substance by the uterine horn adjacent to the ovary. The nature of this substance is at present unknown. One substance produced by the uterus, the prostaglandin $F_{2\alpha}$, is capable of causing luteal regression and it has been demonstrated to be present in increased amounts in uterine venous plasma at the time of luteal regression. It does seem extremely likely that prostaglandin $F_{2\alpha}$ is the luteolytic factor. If this

is the case, then we are provided with a substance for synchronising oestrus in animals in a completely physiological manner. If prostaglandin $F_{2\alpha}$ is injected into the uterus it will be picked up by the uterine vein, carried across to the utero-ovarian artery and from there proceed to regress the corpus luteum. If it is simple to inject, and provided that repeated injections are not necessary, this may be a valuable way of synchronising oestrus and of improving fertility.

7. The lack of suitable methods for storing ram semen, particularly for frozen storage and the lowered fertility to artificial insemination at controlled oestrus, are two important problems preventing introduction of artificial insemination on a practical basis in sheep. More work is needed to determine those combinations of dilution rates and freezing rates which will return the maximum number of viable cells.
8. Sheep production is undergoing changes by such techniques as artificial insemination, superovulation and induction of oestrus during a normally anoestrous period either by light control or hormone therapy. Further changes are expected with the development of techniques for ova transplantation. Very high output of genetically superior lambs derived from a single ewe could be achieved by superovulation, and fertilization in the test tube, followed by ova transplantation into less genetically useful recipient ewes. Fertilized sheep eggs will remain viable when transferred to the

fallopian tube of a rabbit for at least 5 days, thus allowing long distant transport of a large number of sheep as fertilized eggs. The egg transfer approach is as yet in the early ~~embryonic~~ stage but it may well eventually develop into a procedure superceding artificial insemination if concerted efforts are made.

9. The incorporation of these new and complex techniques into the fabric of agriculture has to be undertaken with considerable skill. It demands the incorporation of the techniques into systems and the careful comparison of these systems with more traditional methods. It may well be that this last stage is the most critical in the chain and the most demanding in terms of applied research.

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